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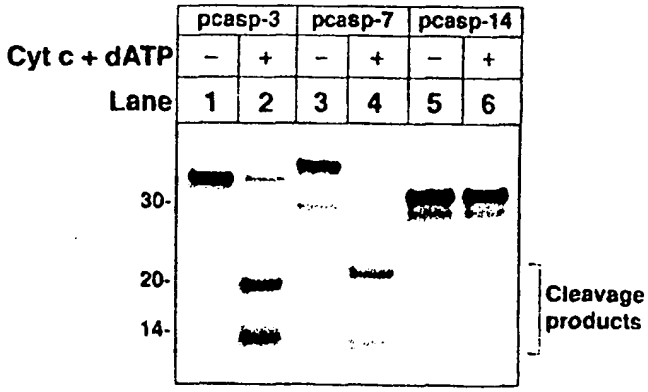

(57) Abstract

The invention relates to an isolated nucleic acid molecule encoding a caspase–14 polypeptide or functional fragment thereof, a vector that contains the nucleic acid molecule and a host cell that contains the vector. The invention also relates to an isolated gene encoding caspase–14, as well as functional fragments thereof. The gene or nucleic acid molecule can include single or double stranded nucleic acids corresponding to coding or non–coding strands of the caspase–14 nucleotide sequence. Isolated caspase–14 polypeptides or functional fragments thereof are also provided, as are antibodies that specifically bind thereto. In addition, the invention relates to methods of identifying compounds that modulate caspase–14 activity.

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CASPASE-14, AN APOPTOTIC PROTEASE, NUCLEIC ACIDS ENCODING AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U. S. Serial No. 09/139,600, filed August 25, 1998, now pending, which application claims the benefit of priority from U. S. Provisional Application Serial No. 60/056,986, now abandoned.

STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under grant AI 35035-1 from the National Institutes of Health. Accordingly, the government has certain rights to this invention.

TECHNICAL FIELD

This invention relates generally to caspase-14, and in particular, to nucleic acids encoding caspase-14, the encoded polypeptides, antibodies thereto, and methods of identifying modulators of caspase-14 activity.

BACKGROUND OF THE INVENTION

Apoptosis, also referred to as physiological cell death or programmed cell death, is a normal physiological process of cell death that plays a critical role in the regulation of tissue homeostasis by ensuring that the rate of new cell accumulation produced by cell division is offset by a commensurate rate of cell loss due to death. Apoptosis can be characterized by morphological changes in the cell, including fragmentation of nuclear chromatin, compaction of cytoplasmic organelles, dilatation of the endoplasmic reticulum, a decrease in cell volume and alterations to the plasma membrane, resulting in the recognition and phagocytosis of apoptotic cells and prevention of an inflammatory response. Disturbances in apoptosis that prevent or delay normal cell turnover can be just as important to the pathogenesis of diseases as are known abnormalities in the regulation of proliferation and the cell cycle. Like cell

division, which is controlled through complex interactions between cell cycle regulatory proteins, apoptosis is similarly regulated under normal circumstances by the interaction of gene products that either induce or inhibit cell death.

The stimuli that regulate the function of these apoptotic gene products include both extracellular and intracellular signals. Either the presence or the removal of a particular stimulus can be sufficient to evoke a positive or negative apoptotic signal. Physiological stimuli that inhibit or reduce the likelihood of apoptosis include, for example, growth factors, extracellular matrix, CD40 ligand, viral gene products, neutral amino acids, zinc, estrogen and androgens. In contrast, stimuli that promote apoptosis include, for example, tumor necrosis factor (TNF), Fas, transforming growth factor β (TGFβ), neurotransmitters, growth factor withdrawal, loss of extracellular matrix attachment, intracellular calcium and glucocorticoids. Other stimuli, including those of environmental and pathogenic origin, also exist and can either induce or inhibit apoptosis. Although apoptosis is mediated by diverse signals and complex interactions of cellular gene products, the results of these interactions ultimately lead into a cell death pathway that is evolutionarily conserved between humans and invertebrates.

Several gene products that modulate the apoptotic process have been identified. Although these products can, in general, be separated into two basic categories, gene products from each category can function to either inhibit or induce apoptosis. One family of gene products is the Bcl-2 family of proteins. Bcl-2 is the best characterized member of this family and inhibits apoptosis when overexpressed in cells. Other members of the Bcl-2 family of proteins include, for example, Bax, Bak, Bcl-x_L, Bcl-x_S and Bad. While some of these proteins can inhibit apoptosis, others can induce apoptosis (for example, Bcl-x_S and Bak, respectively).

A second family of gene products, the caspase family, is related genetically to the *C. elegans* ced-3 gene product, which is required for apoptosis in the roundworm, *C. elegans*. The caspase family includes, for example, caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9 and caspase-10. Among the common features of the caspase gene products is that 1) they are cysteine proteases with specificity for substrate cleavage at Asp-X bonds,

where "X" is an amino acid; 2) they share a conserved pentapeptide sequence within the active site; and 3) they are synthesized as proenzymes that require proteolytic cleavage at specific aspartate residues for activation of protease activity. Cleavage of the proenzyme produces two polypeptide protease subunits, which combine non-covalently to form a tetramer comprised of two heterodimers. Although these proteases, when expressed in cells, induce apoptosis, several alternative structural forms of these proteases, such as caspase-1δ (ICEδ), caspase-1ε (ICEε), caspase-2_S (ICH-1_S), caspase-6β (Mch2β) and caspase-7β (Mch3β), inhibit apoptosis.

In addition to the Bcl-2 and caspase families, which play a role in apoptosis in mammalian cells, other gene products are important in mammalian apoptosis. For example, in addition to ced-3, another *C. elegans* gene product, ced-4, is required for apoptosis in *C. elegans*. Apaf-1, a human protein homologous to ced-4, binds cytochrome c and may activate caspase-3, leading to apoptosis. In addition, another protein, casper, while not a caspase, has sequence similarity to caspase-8 throughout its length and interacts with caspase-8 and caspase-3 through distinct domains. Overexpression of casper in mammalian cells induces apoptosis.

It is uncertain whether other genes encode members of either of the Bcl-2 or caspase gene families and, if so, what role they play in the apoptotic pathway. It also is unclear what physiological control mechanisms regulate apoptosis and how the apoptotic pathways interact with other physiological processes. For example, it has been suggested that cytotoxic T lymphocytes mediate their destructive function by inducing apoptosis in their target cells.

The process of apoptosis maintains tissue homeostasis in various physiological processes, including embryonic development, immune cell regulation and normal cell turnover. It follows that the loss of apoptosis can lead to a variety of pathological disease states. For example, the inappropriate loss of apoptosis can lead to the pathological accumulation of self-reactive lymphocytes such as those occurring in association with many autoimmune diseases. Inappropriate loss of apoptosis also can lead to the accumulation of virally infected cells and of hyperproliferative cells such as tumor cells. Similarly, the inappropriate activation of apoptosis can contribute to a

variety of pathological disease states including, for example, acquired immunodeficiency syndrome (AIDS), neurodegenerative diseases and ischemic injury. Treatments that are specifically designed to modulate the apoptotic pathways in these and other pathological conditions can change the natural progression of many of these diseases.

Thus, there exists a need to identify apoptotic genes and their gene products and for methods of modulating apoptosis for the therapeutic treatment of human diseases. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The invention generally provides caspase-14. In one aspect, the invention provides an isolated nucleic acid molecule encoding a caspase-14 polypeptide or a functional fragment thereof. Nucleic acid and amino acid sequences of caspase-14 are provided. The invention also provides caspase-14 polypeptides or a functional fragment thereof.

In another aspect, a vector that contains the nucleic acid molecule and a host cell that contains the vector is also provided. Also provided is an expression vector comprising the nucleic acid molecule encoding caspase-14 that is operatively linked to a promoter.

In other aspects, an isolated caspase-14 polypeptide and functional fragment thereof are also provided, as are antibodies that specifically bind thereto. In addition, the invention provides methods of identifying compounds that modulate caspase-14 activity comprising: (a) contacting a sample containing a caspase-14 polypeptide or functional fragment thereof with a test compound, and thereafter (b) determining the activity of caspase-14 polypeptide or functional fragment thereof.

Methods are also provided for identifying inhibitors and enhancers of caspase-14 activity, comprising: (a) contacting an activated caspase-14 polypeptide with a substrate in the presence of a test compound under conditions in which the caspase-14 processes the substrate in the absence of the test compound; and thereafter (b) detecting

increased or decreased substrate turnover, wherein increased substrate turnover indicates the presence of an enhancer and wherein decreased substrate turnover indicates the presence of an inhibitor.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, the various references set forth below that describe in more detail certain procedures or compositions (*e.g.*, plasmids, etc.), and are therefore incorporated herein, by reference, in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of caspase-14. The double-stranded nucleotide sequence is shown, with nucleotide position numbers shown on the left and right sides. The encoded amino acids are shown below each row of nucleotides, with stop codons designated by an asterisk. The start codon, which is shown at nucleotide positions 61 to 63, was identified based on the presence of a stop codon upstream of this position (nucleotide positions 49 to 51). Amino acid position numbers are shown on the right side of the sequence, with the letter "a" shown on the left side of the amino acid sequence and the initial methionine designated position 1. The open reading frame encodes caspase-14, which is 257 amino acids in length, and ends with a stop codon at nucleotide positions 832 to 834.

Figure 2 illustrates the amino acid sequence analysis and primary structure of caspase-14. Figure 2A shows a colinear alignment of the predicted amino acid sequence of procaspase-14 with the amino acid sequence of 8 other known caspases. Noncontiguous sequences of caspase-14 (SEQ ID NOS:____), Mch5 (caspase-8; SEQ ID NOS: ____), Mch3 (caspase-7; SEQ ID NOS: ____), Mch2 (caspase-6; SEQ ID NOS: ____), CPP32 (caspase-3; SEQ ID NOS: ____), ICE (caspase-1; SEQ ID NOS: ____), and ICH-1 (caspase-2; SEQ ID NOS: ____) are shown. The amino acid position of the first amino acid shown in the respective proteins is indicated on the left. Figure 2B depicts the primary structure of procaspase-14

represented by a bar diagram. The active site QACRG pentapeptide and potential aspartate processing sites are indicated.

Figure 3 is a scanned image of an autoradiogram representing a Northern blot of the tissue distribution in mouse of caspase-14 mRNA.

Figure 4 is a bar diagram representing the ability of procaspase-14 overexpression in MCF-7 cells to initiate apoptosis.

Figures 5A-C are scanned images of autoradiograms representing SDS-PAGE analysis of the expression and processing of procaspase-14.

Figure 6 is a scanned image of an autoradiogram representing SDS-PAGE analysis of the processing of procaspase-14 in S-100 extracts.

Figure 7 illustrates the nucleotide sequence (SEQ ID NO:4) and the deduced amino acid sequence (SEQ ID NO:5) of caspase-14. The nucleotide sequence is shown, with nucleotide position numbers shown on the right side. The encoded amino acids are shown below each row of nucleotides, with stop codons designated by an asterisk. The start codon, which is shown at nucleotide positions 49 to 51. Amino acid position numbers are shown below the sequence and the initial methionine designated position 1. The open reading frame encodes a caspase-14, which is 242 amino acids in length, and ends with a stop codon at nucleotide positions 775-777.

Figure 8 illustrates the nucleotide sequence (SEQ ID NO:6) and the deduced amino acid sequence (SEQ ID NO:7) of a splice variant of human caspase-14. The nucleotide sequence is shown, with nucleotide position numbers shown to the right side. The encoded amino acids are shown below each row of nucleotides, with stop codon designated by an asterisk. The start codon, which is shown at nucleotide positions 49 to 51. Amino acid position numbers are shown below the sequence and the initial methionine designated position 1. The open reading frame encodes a caspase-14, which is 230 amino acids in length. The active site is at amino acid positions 130-134 and the cleavage between the large and small subunit is at positions 146 and 147. This sequence differs from that in Figure 7 in that there is an intronic insertion at position 568 which results in a shift in the reading frame and a shorter protein.

Figure 9 illustrates the nucleotide sequence (SEQ ID NO:8) and the deduced amino acid sequence (SEQ ID NO:9) of a splice variant of human caspase-14. The nucleotide sequence is shown, with nucleotide position numbers shown to the right side. The encoded amino acids are shown below each row of nucleotides, with stop codon designated by an asterisk. The start codon, which is shown at nucleotide positions 49 to 51. Amino acid position numbers are shown below the sequence and the initial methionine designated position 1. The open reading frame encodes a caspase-14, which is 214 amino acids in length. The active site is at amino acid positions 102-106 and the cleavage site separating the large and small subunit is between positions 118 and 119. This sequence differs from that in Figure 7 in that it has an internal deletion at position 151, which results in a shorter protein.

Figure 10 is an identity comparison between the mouse and human caspase-14 polypeptide sequences as described in Example 1. The mouse sequence is represented on the top line and the human sequence is represented on the bottom line.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a cell death specific protease, termed caspase-14, which is a member of the caspase family of proteases that includes, for example, with alternate designations in parentheses, caspase-1 (ICE, interleukin-1-β converting enzyme), caspase-2_L (ICH-1_L), caspase-2_S (ICH-1_S), caspase-3 (CPP32), caspase-4 (TX, ICH-2, ICE_{rel}-II), caspase-5 (ICE_{rel}-III, TY), caspase-6 (Mch2), caspase-7 (Mch3, ICE-LAP3, CMH-1), caspase-8 (Mch5, MACH, FLICE), caspase-9 (Mch6, ICE-LAP6) and caspase-10 (Mch4). Similar to other caspases, caspase-14 is produced as a proenzyme and becomes active following proteolytic cleavage into a larger and smaller subunit. The two subunits form heterodimers that associate with each other into a heterotetrameric active complex, which induces apoptosis. Substrate specificity uniquely requires an aspartic acid residue in the P1 position of the substrate binding site with a small, preferably hydrophobic, residue in the P1' position.

A nucleic acid molecule (SEQ ID NO:1), which encodes a caspase-14 polypeptide (SEQ ID NO:2) was identified and isolated based on identifying an

expressed sequence tag (EST) having GenBank accession number AA103647, a sequence of 483 nucleotides in length. The EST was identified during a homology search of the GenBank database using a query nucleotide sequence based on caspase-3 and caspase-6 coding sequences (see Example 1). The mouse cell clone that contained the sequence from which the EST was derived was obtained from IMAGE Consortium. It was discovered that the clone, which had been only partially and inaccurately sequenced, contained a nucleotide sequence (SEQ ID NO:1) encoding caspase-14. This caspase had the highest homology with procaspase-3 (32% identity) and procaspase-7 (31% identity). The differences between the EST and the corresponding sequence of the coding strand shown in Figure 1 (SEQ ID NO:1) include, for example, that the coding strand shown in Figure 1 (SEQ ID NO:1) contains a cytosine at nucleotide position 13 and guanines at nucleotide positions 54 and 164, while the EST contains nothing at the corresponding positions.

The invention also provides additional caspase-14 nucleic acid molecules such as (SEQ ID NO:4), that encodes human caspase-14 (SEQ ID NO:5), which is preferentially expressed in keratenocytes as determined by RT-PCR in normal keratenocytes as well as transformed keratenocytes such as the A431 cell line. As demonstrated by Figure 7, the nucleotide sequence is 777 nucleotides, while the encoded polypeptide is 242 amino acids in length. This human sequence was identified as demonstrated in Example 1, using nested PCR primers corresponding to the mouse sequence (SEQ ID NO:1). Further provided are splice variant isoforms of SEQ ID NO:4. In one embodiment such isoforms are provided by nucleic acid molecules illustrated in Figure 8 (SEQ ID NO:6) and Figure 9 (SEQ ID NO:8) as well as their respectively encoded human caspase-14 polypeptides (SEQ ID NOS:7 and 9). Such splice variants are identified using high stringency probes derived from SEQ ID NOS:1 or 4.

The invention provides isolated caspase-14 polypeptides such as SEQ ID NOS:2 or 5 as well as splice variants thereof (e.g., SEQ ID NOS:7 and 9). The term "isolated" means in a form that is relatively free from contaminating lipids, unrelated polypeptides, nucleic acids and other cellular material normally associated with the

polypeptide in the cell and at least about 30% of the total material. In another embodiment of the invention, the isolated caspase-14 polypeptide is about 50% of the total material. In another embodiment of the invention, the isolated caspase-14 polypeptide is about 70% of the total material. In another embodiment of the invention, the isolated caspase-14 polypeptide is about 90% of the total material. In yet another embodiment of the invention, the isolated caspase-14 polypeptide is greater than about 95% of the total material. Thus, an isolated polypeptide of the invention is one that is in a form that is different from the naturally occurring state.

Exemplary polypeptides of the invention are the isolated mouse caspase-14 polypeptide 257 amino acids in length and shown as SEQ ID NO:2 (Figure 1), the isolated human caspase-14 polypeptide 242 amino acids in length and shown as SEQ ID NO:5 (Figure 7), and the isolated human caspase-14 isoforms shown as SEQ ID NOS:7 and 9. The invention further provides an isolated caspase-14 polypeptide, which has greater than about 33% amino acid sequence identity with SEQ ID NOS:2, 5 or their respective isoforms. In other embodiments of the invention, the polypeptide has generally greater than about 50% or 60% amino acid sequence identity with SEQ ID NOS:2, 5 or their respective splice variant isoforms. In yet other embodiments of the invention, the polypeptide has generally greater than about 70% or 80% amino acid sequence identity with SEQ ID NOS:2, 5, or their respective isoforms. Such amino acid sequence identity may be determined by standard methodologies, including use of the National Center for Biotechnology Information BLAST search methodology available at www.ncbi.nlm.nih.gov. The identity methodologies preferred are those described in U.S. Patent 5,691,179 and Altschul et al., Nucleic Acids Res. 25:3389-3402, 1997 all of which are incorporated herein by reference. Most preferred and that which is used to calculate percentages herein is the pileUP algorithm as described in Example 1.

A caspase-14 polypeptide includes polypeptides having substitutions of conserved and non-essential amino acids of SEQ ID NOS:2 or 5 and, generally includes, for example, mammalian homologues of SEQ ID NOS:2 or 5 such as rat or other mammalian caspase-14. A caspase-14 polypeptide also can include polypeptides having related but different sequences, provided the polypeptide has at least one

functional activity of SEQ ID NOS:2 or 5, such as protease activity. For example, splice variant isoforms of SEQ ID NOS:1 or 4, such as those provided by SEQ ID NOS:6 and 8 are included in such a definition. Therefore, it should be understood that when referencing the various polypeptides and nucleic acid molecules, the splice variant isoforms and homologous sequences thereof are implicated as well. Accordingly, with regard to fragments, the specific disclaimers to contiguous sequences found in the prior art as to SEQ ID NOS:1, 2, 4, and 5, also includes the identical sequences found in SEQ ID NOS:6-9.

It is understood that limited modifications may be made to a caspase-14 polypeptide without destroying its biological function and that only a portion of the entire primary structure may be required in order to effect activity. Thus, for example, minor modifications of SEQ ID NOS:2 or 5 provide examples of caspase-14 polypeptides. Such minor modifications may result in polypeptides that have substantially equivalent or enhanced function as compared to SEQ ID NOS:2 or 5. These modifications may be deliberate, such as through site-directed mutagenesis, or may be accidental, such as through mutation in hosts that are caspase-14 producers. It also is understood that allelic variants and splice variants of caspase-14 are caspase-14 polypeptides encompassed within the invention.

In addition, the invention provides a functional fragment of SEQ ID NOS:2 or 5 or splice variants thereof. A functional fragment of SEQ ID NOS:2 or 5 is defined structurally and functionally in that it has the same contiguous sequence as a portion of SEQ ID NOS:2 or 5 and at least one biological activity characteristic of caspase-14. A functional fragment of SEQ ID NOS:2, 5, or a splice variant thereof comprises at least 8 contiguous residues of SEQ ID NOS:2, 5, or a splice variant thereof. In other embodiments of the invention, a functional fragment of SEQ ID NOS:2, 5, or a splice variant thereof, comprises an amino acid sequence of at least 10 or 12 contiguous residues. In other embodiments of the invention, a functional fragment of SEQ ID NOS:2 or 5 comprises an amino acid sequence of at least 15 or 20 contiguous residues. In other embodiments of the invention, a functional fragment of SEQ ID NOS:2, 5, or splice variants thereof, comprises an amino acid sequence of at

least 25 or 30 contiguous residues. In another embodiment of the invention, a functional fragment of SEQ ID NOS:2, 5, or splice variants thereof comprises an amino acid sequence of at least 50 contiguous residues. In yet other embodiments of the invention, a functional fragment of SEQ ID NO:2 comprises an amino acid sequence of at least 6 or 7 contiguous residues of SEQ ID NO:2, provided that such sequence does not include amino acid positions 132 to 138 or 134 to 139 of SEQ ID NO:2 or the homologous sequences from splice variant forms. In yet other embodiments of the invention, a functional fragment of SEQ ID NO:5 comprises an amino acid sequence of at least 6 or 7 contiguous residues of SEQ ID NO:2, provided that such sequence does not include amino acid positions 128 to 133 or 130 to 135 of SEQ ID NO:5 or the homologous sequences from splice variant forms. However, an amino acid sequence that consists of the identical amino acid sequence encoded by the EST having GenBank accession number AA103647, or any contiguous portion thereof, is not a functional fragment of SEQ ID NOS:2 or 5 encompassed within the invention. Similarly, a contiguous portion of caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10 or ced-3 is not a functional fragment of SEQ ID NOS:2, 5, 7, or 9, encompassed within the invention. A biological activity of a functional fragment of SEQ ID NOS:2 or 5 is an activity of caspase-14 and can be, for example, the ability to bind a ligand, have protease or other enzymatic activity, enhance or inhibit apoptosis or bind or induce the production of an anti-caspase-14 antibody.

The invention also provides a functional fragment of a caspase-14 polypeptide. Such a functional fragment is defined structurally and functionally in that it has amino acid sequence identity to a portion of SEQ ID NOS:2 or 5, as described below, and has at least one biological activity of caspase-14, as described above. A functional fragment of a caspase-14 polypeptide that does not include QACRG (SEQ ID NO:3; amino acid positions 134 to 138 of SEQ ID NO:2; amino acid positions 130-134 of SEQ ID NO:5 or the homologous portions of a splice variant) has several embodiments. In one embodiment such a functional fragment comprises at least 10 amino acids and has at least about 70% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In other embodiments, such a functional fragment comprises

at least 10 amino acids and has at least about 75% or 80% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In other embodiments, such a functional fragment comprises at least 10 amino acids and has at least about 85% or 90% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In another embodiment, such a functional fragment comprises at least 25 amino acids and has at least about 65% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In other embodiments, such a functional fragment comprises at least 25 amino acids and has at least about 70% or 75% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In other embodiments, such a functional fragment comprises at least 25 amino acids and has at least about 80% or 85% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In other embodiments, such a functional fragment comprises at least 40 amino acids and has at least about 50% or 60% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In yet other embodiments, such a functional fragment comprises at least 40 amino acids and has at least about 70% or 80% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. The aforementioned identities being calculated with the pileUP algorithm as defined in Example 1.

In comparison, a functional fragment of a caspase-14 polypeptide that includes QACRG (SEQ ID NO:3; amino acid positions 134 to 138 of SEQ ID NO:2; amino acid positions 130 to 134 of SEQ ID NO:5 or homologous portions of splice variants) comprises at least about 13 amino acids and has greater than about 92% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In other embodiments of the invention, such a functional fragment comprises at least about 13 amino acids and has greater than about 93% or 95% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In another embodiment of the invention, such a functional fragment comprises at least about 13 amino acids and has greater than about 98% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In another embodiment of the invention, such a functional fragment comprises at least about 25 amino acids and has greater than about 72% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In other embodiments of the invention, such a

functional fragment or comprises at least about 25 amino acids and has greater than about 75% or 80% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. In yet other embodiments of the invention, such a functional fragment or comprises at least about 25 amino acids and has greater than about 85% or 90% or 95% amino acid sequence identity with a portion of SEQ ID NOS:2, 5, 7, or 9. A fragment that consists of the identical amino acid sequence encoded by the EST having GenBank accession number AA103647, or any contiguous portion thereof, or of any contiguous portion of caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10 or ced-3, is not considered a functional fragment of a caspase-14 polypeptide.

It is understood that functional fragments of a caspase-14 polypeptide include fragments with substitutions of conserved and non-essential amino acids of portions of SEQ ID NO:2 and, therefore, include, for example, fragments of eukaryotic homologs of SEQ ID NO:2 such as fragments of yeast or *Drosophila* or *C. elegans* caspase-14. However, it also is understood, that contiguous fragments of caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10 or ced-3 polypeptides, for example, are not functional fragments of a caspase-14 polypeptide encompassed within the invention.

SEQ ID NOS:2 or 5 are inactive proenzymes, which are proteolytically cleaved to form a large subunit and a small subunit, which provide examples of functional fragments of SEQ ID NOS:2 or 5. Proteolytic cleavage of SEQ ID NO:2 occurs between the aspartic acid and glutamic acid residues of the shown in Figure 2. Thus, cleavage occurs between amino acid positions 156 and 157 of SEQ ID NO:2 and between amino acid positions 162 and 163 of SEQ ID NO:2, resulting in a large subunit comprising amino acid positions 1 to 156 of SEQ ID NO:2 and a small subunit comprising amino acid positions 163 to 257 of SEQ ID NO:2. Proteolytic cleavage of SEQ ID NO:5 occurs between the aspartic acid and glutamic acid residues at positions 146 and 147 of SEQ ID NO:5, resulting in a large subunit comprising amino acids 1-146 of SEQ ID NO:5 and a small subunit comprising amino acid positions 147-242 of SEQ ID NO:5. Other caspase-14 polypeptides, which are inactive proenzymes, also are

proteolytically cleaved to form large subunits and small subunits. which provide examples of functional fragments of caspase-14. The large and small subunits of a caspase-14 polypeptide can combine non-covalently to produce a heterotetramer having apoptotic activity.

If desired, the large subunit of a caspase-14 polypeptide can be combined with a small subunit of another caspase polypeptide such as caspase-3 (CPP32) to form an apoptotic complex, or the small subunit of a caspase-14 polypeptide can be combined with a large subunit of another caspase protein such as caspase-3 (CPP32) to form an apoptotic complex. Such complexes can be formed *in vitro*, in cells in culture, or *in vivo* by heterodimerization of the large and small subunits.

The activity of a caspase-14 polypeptide or functional fragment thereof can be measured enzymatically (see Example 2). If desired, a caspase-14 polypeptide or functional fragment thereof can be attached to a second molecule such as, for example, a protein, carbohydrate, lipid or chemical moiety. For example, a caspase-14 polypeptide or functional fragment thereof can be fused to a heterologous protein such as a fusion protein that retains caspase-14 enzymatic or other biological activity and has a characteristic of the heterologous protein.

An isolated caspase-14 polypeptide or functional fragment thereof can be obtained by a variety of methods known in the art. For example, a caspase-14 polypeptide can be isolated by biochemical methods such as affinity chromatography. Affinity matrices that can be used for caspase-14 isolation can be a solid phase having attached thereto anti-caspase-14 monoclonal or polyclonal antibodies prepared against a caspase-14 polypeptide or a functional fragment thereof comprising a caspase-14 epitope. Alternatively, ligands such as substrate analogues or enzymatic inhibitors of caspase-14 can be used as affinity matrices to isolate a caspase-14 polypeptide or functional fragment thereof that binds the ligand.

Other biochemical methods for isolating a caspase-14 polypeptide or functional fragment thereof include preparative gel electrophoresis, gel filtration, affinity chromatography, ion exchange and reversed phase chromatography, chromatofocusing, isoelectric focusing and sucrose or glycerol density gradients

(Deutscher, *Methods in Enzymology: Guide to Protein Purification*, Vol. 182, Academic Press, Inc., San Diego (1990), Chapter 38; Balch et al., *Methods in Enzymology*, Vol. 257, Academic Press, Inc., San Diego (1995), Chapter 8). For example, a caspase-14 polypeptide or functional fragment thereof can be isolated by preparative polyacrylamide gel electrophoresis and elution by diffusion or electroelution (Deutscher, *supra*, 1990, Chapter 33). Continuous elution gel electrophoresis using a system such as the Model 491 Prep Cell (BioRad, Hercules, CA) can be used to isolate a caspase-14 polypeptide or functional fragment thereof. If desired, continuous elution gel electrophoresis can be combined with further purification steps such as liquid phase preparative isoelectric focusing using, for example, the Rotofor system (BioRad).

A caspase-14 polypeptide or functional fragment thereof also can be produced by chemical synthesis, for example, by the solid phase peptide synthesis method (Merrifield et al., *J. Am. Chem. Soc. 85*:2149 (1964)). Standard solution methods well known in the art also can be used to synthesize a caspase-14 polypeptide or functional fragment thereof (Bodanszky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin (1984); Bodanszky, *Peptide Chemistry*, Springer-Verlag, Berlin (1993)). A newly synthesized caspase-14 polypeptide or functional fragment thereof can be isolated, for example, by high performance liquid chromatography and can be characterized using mass spectrometry or amino acid sequence analysis.

A caspase-14 polypeptide or functional fragment thereof also can be produced by recombinant DNA methods. Accordingly, the invention provides a nucleic acid molecule encoding a caspase-14 polypeptide or functional fragment thereof. Such a nucleic acid molecule can be cloned into an appropriate vector for propagation, manipulation or expression as desired. Such a vector is commercially available or can be constructed by those skilled in the art and contains expression elements necessary for the transcription, translation, regulation, and, if desired, sorting of the caspase-14 polypeptide or functional fragment thereof. The selected vector also can be used in a procaryotic or eukaryotic host system, as appropriate, provided the expression and regulatory elements are of compatible origin. A recombinant caspase-14 polypeptide or

functional fragment thereof produced in a host cell or secreted from the cell can be isolated using, for example, an anti-caspase-14 antibody, as described herein.

Caspase-14 may be expressed in a variety of host organisms. In certain embodiments, caspase-14 is produced in bacteria, such as *E. coli*, or mammalian cells (*e.g.*, CHO and COS-7), for which many expression vectors have been developed and are available. Other suitable host organisms include other bacterial species, and eukaryotes, such as yeast (*e.g., Saccharomyces cerevisiae*), and insect cells (*e.g.*, Sf9).

In one embodiment, a DNA sequence encoding caspase-14 is introduced into an expression vector appropriate for the host cell. In certain embodiments, caspase-14 is inserted into a vector such that a fusion protein is produced. The caspase-14 sequence is derived as described herein. As discussed above, the sequence may contain alternative codons for each amino acid with multiple codons. The alternative codons can be chosen as "optimal" for the host species. Restriction sites are typically incorporated into the primer sequences and are chosen with regard to the cloning site of the vector. If necessary, translational initiation and termination codons can be engineered into the primer sequences.

At a minimum, the vector will contain a promoter sequence. As used herein, a "promoter" refers to a nucleotide sequence that contains elements that direct the transcription of a linked gene. At a minimum, a promoter contains an RNA polymerase binding site. More typically, in eukaryotes, promoter sequences contain binding sites for other transcriptional factors that control the rate and timing of gene expression. Such sites include TATA box, CAAT box, POU box, AP1 binding site, and the like. Promoter regions may also contain enhancer elements. When a promoter is linked to a gene so as to enable transcription of the gene, it is "operatively linked".

Other regulatory sequences may be included. Such sequences include a transcription termination sequence, secretion signal sequence, origin of replication, selectable marker, and the like. The regulatory sequences are operationally associated with one another to allow transcription or translation.

The expression vectors used herein include a promoter designed for expression of the proteins in a host cell (*e.g.*, bacterial). Suitable promoters are widely

available and are well known in the art. Inducible or constitutive promoters are preferred. Such promoters for expression in bacteria include promoters from the T7 phage and other phages, such as T3, T5, and SP6, and the *trp*, *lpp*, and *lac* operons. Hybrid promoters (*see*, U.S. Patent No. 4,551,433), such as *tac* and *trc*, may also be used. Promoters for expression in eukaryotic cells include the P10 or polyhedron gene promoter of baculovirus/insect cell expression systems (*see*, *e.g.*, U.S. Patent Nos. 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784), MMTV LTR, CMV IE promoter, RSV LTR, SV40, metallothionein promoter (*see*, *e.g.*, U.S. Patent No. 4,870,009), ecdysone response element system, tetracycline-reversible silencing system (tet-on, tet-off), and the like.

The promoter controlling transcription of caspase-14 may itself be controlled by a repressor. In some systems, the promoter can be derepressed by altering the physiological conditions of the cell, for example, by the addition of a molecule that competitively binds the repressor, or by altering the temperature of the growth media. Preferred repressor proteins include, but are not limited to the *E. coli* lacI repressor responsive to IPTG induction, the temperature sensitive λcI857 repressor, and the like.

In other optional embodiments, the vector also includes a transcription termination sequence. A "transcription terminator region" has either a sequence that provides a signal that terminates transcription by the polymerase that recognizes the selected promoter and/or a signal sequence for polyadenylation.

In one aspect, the vector is capable of replication in the host cells. Thus, when the host cell is a bacterium, the vector preferably contains a bacterial origin of replication. Bacterial origins of replication include the f1-ori and col E1 origins of replication, especially the ori derived from pUC plasmids. In yeast, ARS or CEN sequences can be used to assure replication. A well-used system in mammalian cells is SV40 ori.

The plasmids also preferably include at least one selectable marker that is functional in the host. A selectable marker gene includes any gene that confers a phenotype on the host that allows transformed cells to be identified and selectively grown. Suitable selectable marker genes for bacterial hosts include the ampicillin

resistance gene (Amp^r), tetracycline resistance gene (Tc^r) and the kanamycin resistance gene (Kan^r). The kanamycin resistance gene is presently preferred. Suitable markers for eukaryotes usually require a complementary deficiency in the host (*e.g.*, thymidine kinase (tk) in tk- hosts). However, drug markers are also available (*e.g.*, G418 resistance and hygromycin resistance).

The sequence of nucleotides encoding caspase-14 may also include a secretion signal, whereby the resulting peptide is a precursor protein processed and secreted. The resulting processed protein may be recovered from the periplasmic space or the fermentation medium. Secretion signals suitable for use are widely available and are well known in the art (von Heijne, *J. Mol. Biol. 184*:99-105, 1985). Prokaryotic and eukaryotic secretion signals that are functional in *E. coli* (or other host) may be employed. The presently preferred secretion signals include, but are not limited to, those encoded by the following *E. coli* genes: pelB (Lei *et al.*, *J. Bacteriol. 169*:4379, 1987), phoA, ompA, ompT, ompF, ompC, beta-lactamase, and alkaline phosphatase.

One skilled in the art will appreciate that there are a wide variety of suitable vectors for expression in bacterial cells and which are readily obtainable. Vectors such as the pET series (Novagen, Madison, WI), the tac and trc series (Pharmacia, Uppsala, Sweden), pTTQ18 (Amersham International plc, England), pACYC 177, pGEX series, and the like are suitable for expression of caspase-14. Baculovirus vectors, such as pBlueBac (*see*, *e.g.*, U.S. Patent Nos. 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784; available from Invitrogen, San Diego) may be used for expression in insect cells, such as *Spodoptera frugiperda* sf9 cells (*see*, U.S. Patent No. 4,745,051). The choice of a bacterial host for the expression of caspase-14 is dictated in part by the vector. Accordingly, commercially available vectors are paired with suitable hosts.

A wide variety of suitable vectors for expression in eukaryotic cells are also available. Such vectors include pCMVLacI, pXT1 (Stratagene Cloning Systems, La Jolla, CA); pCDNA series, pREP series, pEBVHis (Invitrogen, Carlsbad, CA). In certain embodiments, the caspase-14 nucleic acid molecule is cloned into a gene targeting vector, such as pMC1neo, a pOG series vector (Stratagene Cloning Systems).

Caspase-14 polypeptides may be isolated by standard methods, such as affinity chromatography, size exclusion chromatography, metal ion chromatography, ionic exchange chromatography, HPLC, and other known protein isolation methods. (*see generally* Ausubel *et al. supra*; Sambrook *et al. supra*). An isolated purified protein gives a single band on SDS-PAGE when stained with Coomassie blue.

In another embodiment, chimeric caspases or protein fusion-caspases can be constructed by standard molecular biological techniques as described by Sambrook *et al.*, *supra*; Ausubel *et al.*, *supra*. Briefly, the region of interest of one caspase can be cloned into a cloning vector and with the aid of restriction enzymes digested such that the nucleic acid sequence of another caspase may be fused thereto, thereby creating a chimeric nucleic acid molecule encoding a chimeric protein. The same procedure can be used to create a caspase fusion protein, however, in this case many vectors are commercially available which contain fusion constructs and allow direct cloning of the insert of interest into the vector in a simple one step process.

Purified caspase-14 fusion proteins may be used in assays to screen for molecules which modulate apoptosis as described in detail *infra*. In further embodiments, these proteins may also be crystallized and subjected to X-ray analysis to determine the 3-dimensional structure or utilized to generate antibodies.

A recombinant caspase-14 polypeptide or functional fragment thereof can be expressed as a fusion protein with a heterologous "tag" for convenient isolation from bacterial or mammalian host proteins. For example, a histidine-tagged recombinant caspase-14 polypeptide can be isolated by nickel-chelate chromatography. Similarly, a glutathione-S-transferase tag or an antigenic tag such as "FLAG," "AU" or a myc epitope tag also can be included in a recombinant caspase-14 polypeptide or functional fragment thereof (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989)). PINPOINT (Promega Corp.; Madison WI) is a commercially available system for expression of a caspase-14 polypeptide or functional fragment thereof as a fusion protein with a heterologous biotinylated peptide.

A functional fragment of a caspase-14 polypeptide also can be produced, for example, by chemical or proteolytic cleavage of an isolated caspase-14 polypeptide. Methods for chemical and proteolytic cleavage and for purification of the resultant polypeptide fragments are well known in the art (Deutscher, *supra*, 1990).

A caspase-14 polypeptide or functional fragment thereof can be part of a heterodimer or a heterotetrameric apoptotic complex. Conversely, a caspase-14 inhibitor such as the large subunit of caspase-14 that lacks the active site QACRG (SEQ ID NO:3; positions 134-138 of SEQ ID NO:2; positions 130-134 of SEQ ID NO:5), for example, can bind the small subunit of caspase-14 and prevent an active protease complex from forming. Thus, a caspase-14 polypeptide or functional fragment thereof can be screened, for example, for apoptotic activity and a caspase-14 inhibitor can be screened for anti-apoptotic activity. Apoptotic activity is the ability either alone, or in combination with another molecule, to produce cell death accompanied by at least one of the morphological or biochemical alterations characteristic of apoptosis. Morphological alterations characteristic of apoptosis are well known in the art and include, for example, condensed and rounded cellular morphology; membrane blebbing; the formation of apoptotic bodies, which are membrane-bound bodies containing cytoplasmic and nuclear components; and condensation of the nucleus, with cytoplasmic organelles being relatively well maintained (Cohen, Gerald, *supra*, 1997; Studzinski (Ed.), *Cell Growth and Apoptosis*, Oxford: Oxford University Press (1995)). Biochemical alterations characteristic of apoptosis also are well known in the art. The classical biochemical alteration characteristic of apoptosis is the appearance of oligonucleosome-sized fragments of DNA, which produce a "ladder" upon agarose gel electrophoresis. This extensive fragmentation can be preceded by an earlier endonucleolytic cleavage of chromatin, producing DNA fragments of about 50 kb to 300 kb in size.

A variety of assays for determining whether a caspase-14 polypeptide or functional fragment thereof has apoptotic activity or whether a caspase-14 inhibitor has anti-apoptotic activity are well known in the art. Such methods include light microscopy for determining the presence of one or more morphological characteristics

of apoptosis, such as condensed or rounded morphology, shrinking and blebbing of the cytoplasm, preservation of structure of cellular organelles including mitochondria, and condensation and margination of chromatin.

A caspase-14 polypeptide or functional fragment thereof or a caspase-14 inhibitor also can be assayed for respective apoptotic or anti-apoptotic activity using terminal deoxytransferase-mediated (TdT) dUTP biotin nick end-labeling (TUNEL) (Gavriel et al., *J. Cell Biol. 119*:493 (1992); Gorczyca et al., *Int. J. Oncol. 1*:639 (1992); Studzinski, *supra,* 1995). APOPTAG (ONCOR, Inc.; Gaithersburg MD) is a commercially available kit for identification of apoptotic cells using digoxygenin labeling. In addition, a caspase-14 polypeptide or functional fragment thereof or a caspase-14 inhibitor can be assayed for respective apoptotic or anti-apoptotic activity by detecting nucleosomal DNA fragments using agarose gel electrophoresis (Studzinski, *supra,* 1995; Gong et al., *Anal. Biochem. 218*:314 (1994)).

DNA filter elution methodology also can be used to detect apoptosis-associated DNA fragmentation and to determine apoptotic or anti-apoptotic activity (Studzinski, *supra,* 1995; Bertrand et al., *Drug Devel. 34*:138 (1995)). Apoptotic or anti-apoptotic activity also can be detected and quantitated by determining an altered mitochondrial to nuclear DNA ratio as described in Tepper et al., *Anal. Biochem. 203*:127 (1992) and Tepper and Studzinski, *J. Cell Biochem. 52*:352 (1993). One skilled in the art understands that these, or other assays for apoptotic or anti-apoptotic activity, can be performed using routine methodology.

In another embodiment, the invention provides antibodies that specifically bind to caspase-14-specific epitopes. Such caspase-14-specific epitopes are present in caspase-14 polypeptides and functional fragments thereof but not in other caspase polypeptides. Antibodies that bind caspase-14-specific epitopes readily are identified by their inability to cross react with other caspases, ced-3 and the like.

A caspase-14 polypeptide or functional fragment thereof can comprise an immunogenic amino acid sequence or, if haptenic, can be conjugated to another molecule to become immunogenic, as described below. Thus, a caspase-14 polypeptide or functional fragment thereof can be useful for eliciting production of an anti-caspase-

14 antibody. In addition, the invention provides a cell line producing an anti-caspase-14 antibody.

As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. With regard to an anti-caspase-14 antibody of the invention, the term "antigen" means a caspase-14 polypeptide or a functional fragment thereof. An anti-caspase-14 antibody, or antigen binding fragment of such an antibody, is characterized by having specific binding activity for a caspase-14-specific epitope of at least about $1 \times 10^5\ M^{-1}$, generally at least about $1 \times 10^6\ M^{-1}$ and preferably at least about $1 \times 10^8\ M^{-1}$. Fab, $F(ab')_2$, Fd and Fv fragments of an anti-caspase-14 antibody, which retain specific binding activity for a caspase-14-specific epitope, are encompassed within the anti-caspase-14 antibody of the invention.

In addition, the term "antibody" as used herein includes naturally occurring antibodies as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric, bifunctional and humanized antibodies, as well as antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains (Huse et al., *Science 246*:1275-1281 (1989)). These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies are well known in the art (Winter and Harris, *Immunol. Today 14*:243-246 (1993); Ward et al., *Nature 341*:544-546 (1989); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1992); Borrabeck, *Antibody Engineering*, 2d ed., Oxford Univ. Press (1995); Hilyard et al., *Protein Engineering: A practical approach*, IRL Press (1992)).

An anti-caspase-14 antibody can be raised using as an immunogen such as, for example, an isolated caspase-14 polypeptide such as SEQ ID NOS:2 or 5, which can be prepared from natural sources or produced recombinantly, as described above, or a functional fragment of a caspase-14 polypeptide, including synthetic peptides, as described above. A non-immunogenic peptide portion of a functional fragment of a

caspase-14 polypeptide can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH), or by expressing the peptide portion as a fusion protein. Various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art (Harlow and Lane, *supra*, 1992).

An anti-caspase-14 antibody is useful, for example, for determining the presence or level of caspase-14 in a tissue sample, which can be a cell lysate or a histological section. The identification of the presence or level of caspase-14 in a sample can be made using well known immunoassay and immunohistochemical methods (Harlow and Lane, *supra*, 1992). In addition, an anti-caspase-14 antibody can be used in a screening assay to identify agents that modulate the activity of caspase-14 or that modulate the binding of caspase-14 to a second protein.

A particularly useful anti-caspase-14 antibody is one that binds a caspase-14 polypeptide, such as SEQ ID NOS:2 or 5, but not to either the large or small subunit cleavage products of the caspase-14 polypeptide, such as amino acid positions 1 to 156 and 163 to 257 of SEQ ID NO:2 or 1 to 146 and 147 to 242 of SEQ ID NO:5, respectively, as well as the corresponding large and small subunits of splice variant isoforms. Similarly, an antibody that binds to either the large subunit or the small subunit of a caspase-14 polypeptide, but not to the other subunit or the caspase-14 polypeptide, as well as an antibody that binds to a heterodimer comprising the large subunit and the small subunit of a caspase-14 polypeptide or a heterotetramer, but not to the caspase-14 polypeptide, is useful. An antibody that binds a caspase-14 polypeptide is useful to isolate caspase-14 from a sample, whereas an antibody that binds the large subunit or the small subunit of a caspase-14 polypeptide is useful to identify samples with caspase-14 processing activity. An antibody that binds a caspase-14 subunit heterodimer or heterotetramer is useful to isolate caspase-14 with apoptotic activity or in a screening assay to identify, for example, an agent that inhibits heterodimer or heterotetramer formation and, therefore, apoptosis. For convenience, reference herein to an anti-caspase-14 antibody generally includes all such antibodies, although the

skilled artisan will recognize that the choice of a particular antibody will depend on the purpose for which the antibody will be used.

A kit incorporating an anti-caspase-14 antibody can be particularly useful. Such a kit can contain, in addition to an anti-caspase-14 antibody, a reaction cocktail that provides the proper conditions for performing the assay, control samples that contain known amounts of caspase-14 or other appropriate caspase-14 antigen recognized by the antibody and, if desired, a second antibody specific for the anti-caspase-14 antibody. Such an assay also can include a simple method for detecting the presence or amount of caspase-14 in a sample that is bound to the anti-caspase-14 antibody.

An anti-caspase-14 antibody, as well as a caspase-14 polypeptide or functional fragment thereof, can be labeled so as to be detectable using methods well known in the art (Hermanson, *Bioconjugate Techniques*, Academic Press (1996); Harlow and Lane, *supra*, 1992). For example, an anti-caspase-14 antibody can be labeled with various detectable moieties including a radiolabel, an enzyme, biotin or a fluorochrome. Reagents for labeling an anti-caspase-14 antibody can be included in a kit containing the antibody or can be purchased separately from a commercial source.

Following contact, for example, of a labeled antibody with a sample such as a tissue homogenate or a histological section of a tissue, specifically bound labeled antibody can be identified by detecting the particular moiety. Alternatively, a labeled second antibody can be used to identify specific binding of an unlabeled anti-caspase-14 antibody. A second antibody generally will be specific for the particular class of the first antibody. For example, if an anti-caspase-14 antibody is of the IgG class, a second antibody will be an anti-IgG antibody. Such second antibodies are readily available from commercial sources. The second antibody can be labeled using a detectable moiety as described above. When a sample is labeled using a second antibody, the sample is first contacted with a first antibody, which is an anti-caspase-14 antibody, then the sample is contacted with the labeled second antibody, which specifically binds to the anti-caspase-14 antibody and results in a labeled sample.

Methods for raising polyclonal antibodies, for example, in a rabbit, goat, mouse or other mammal, are well known in the art. In addition, monoclonal antibodies can be obtained using methods that are well known and routine in the art (Harlow and Lane, *supra*, 1992). For example, spleen cells from a caspase-14-immunized mammal can be fused to an appropriate myeloma cell line such as SP/02 myeloma cells to produce hybridoma cells. Cloned hybridoma cell lines can be screened using a labeled caspase-14 polypeptide or functional fragment thereof to identify clones that secrete anti-caspase-14 monoclonal antibodies having the desired specificity. Hybridomas expressing anti-caspase-14 monoclonal antibodies having a desirable specificity and affinity can be isolated and utilized as a continuous source of the antibodies, which are useful, for example, for preparing standardized kits as described above. Similarly, a recombinant phage that expresses, for example, a single chain anti-caspase-14 also provides a monoclonal antibody that can used for preparing standardized kits.

A monoclonal anti-caspase-14 antibody can be used to prepare anti-idiotypic antibodies, which present an epitope that mimics a caspase-14-specific epitope recognized by the monoclonal antibody used to prepare the anti-idiotypic antibodies. Where the epitope mimicked includes, for example, a portion of the caspase-14 catalytic domain, the anti-idiotypic antibody can act as a competitor of caspase-14 and, therefore, can be useful for reducing the level of activity of caspase-14 and, consequently, the level of apoptotic activity. Thus, the invention further provides an anti-idiotypic anti-caspase-14 antibody, which mimics a caspase-14-specific epitope, such as an epitope of SEQ ID NOS:2, 5, 7, or 9, an epitope of the large or small subunit of a caspase-14 polypeptide or an epitope of a caspase-14 heterodimer or heterotetramer.

The invention also provides an isolated nucleic acid molecule encoding a caspase-14 polypeptide or functional fragment thereof. The term "isolated" means in a form that is relatively free from contaminating lipids, polypeptides, unrelated nucleic acid molecules and other cellular material normally associated with the nucleic acid molecule in the cell and at least 30% of the total material. In other embodiments of the invention, the nucleic acid molecule is 50% or 70% of the total material. In other embodiments of the invention, the nucleic acid molecule is 90% or 95% of the total

material. In yet another embodiment of the invention, the nucleic acid molecule is greater than 95% of the total material. Thus, an isolated nucleic acid molecule of the invention is one that is in a form that is different from the naturally occurring state.

One exemplary nucleic acid molecule of the invention is provided by SEQ ID NO:1, which is 850 nucleotides in length and encodes SEQ ID NO:2 (see Figure 1). Another exemplary nucleic acid molecule is provided by SEQ ID NO:4, which is 777 nucleotides in length and encodes SEQ ID NO:5 (see Figure 7). Additional nucleic acid molecules of the invention are those that have an oligonucleotide or polynucleotide sequence that encodes SEQ ID NOS:2 or 5 or a functional fragment thereof. In addition, the invention provides nucleic acid molecules that have an oligonucleotide or polynucleotide sequence that encodes a caspase-14 polypeptide or a functional fragment thereof such as SEQ ID NOS:6 and 8.

Such an oligonucleotide or polynucleotide sequence also can be useful, for example, as a probe or a PCR primer. Such probes can be used to screen a genomic DNA library or a cDNA library to obtain other nucleic acid molecules encoding caspase-14 polypeptides or to diagnose a disease associated with enhanced or inhibited apoptosis (see below). Thus, the invention provides oligonucleotide sequences that comprise at least 12 contiguous nucleotides of SEQ ID NOS:1, 4, 6, or 8. In other embodiments, the invention provides oligonucleotide sequences that comprise at least 15, 18 or 21 contiguous nucleotides of SEQ ID NOS:1, 4, 6, or 8. In another embodiment, the invention provides a nucleic acid molecule encoding SEQ ID NOS:2, 5, or splice variants thereof. In yet another embodiment, the invention provides a nucleic acid molecule encoding a caspase-14 polypeptide. Oligonucleotide sequences consisting of nucleotide positions 454 to 474 or positions 460 to 477 of SEQ ID NO:1, or nucleotide positions 430 to 450 or positions 436 to 453 of SEQ ID NO:4 and/or homologous positions of splice variant forms, or any contiguous portion thereof, however, are not encompassed within the nucleic acid molecules of the invention. Similarly, nucleic acid molecules that consist of the expressed sequence tag having GenBank accession number AA103647, or any contiguous portion thereof, also are not encompassed within the nucleic acid molecules of the invention.

In another embodiment, the invention provides an isolated gene encoding caspase-14, as well as functional fragments of a caspase-14 gene. A gene encoding caspase-14 can be obtained by screening a genomic library using, for example, an oligonucleotide or polynucleotide sequence of SEQ ID NOS:1, 4, 6, or 8 as a probe, as discussed above. Methods of preparing genomic libraries are known in the art (Perbal, Bernard, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, Inc. (1988), ch. 17, and the various references cited therein).

In addition, nucleic acid molecules that do not encode a caspase-14 gene product but, instead, are regulatory elements are considered part of the gene encoding caspase-14, particularly functional fragments of a caspase-14 gene. Specific examples of such functional fragments of a caspase-14 gene include promoters, enhancers and other gene expression regulatory elements present in a caspase-14 encoding gene. Thus, upon obtaining a caspase-14 gene, as described above, regulatory elements present in the caspase-14 gene can be identified using routine methods.

To identify sequences having homology to the caspase family of proteases, nucleic acid molecules encoding apoptotic cysteine proteases can be enriched by PCR amplification of a cDNA library using a primer designed to encompass homologous regions in nucleic acid sequences that encode known caspase protease family members. The enriched library can be further amplified by PCR using a primer with sequences having homology to the putative novel protease cDNA but not to the other caspase family of proteases. For example, to obtain a caspase-14 polypeptide, such as a mammalian homologue of SEQ ID NOS:2, 5, 7, or 9, a primer with sequences homologous to SEQ ID NOS:1, 4, 6, or 8, respectively, but not to the other caspases can be used.

As searching a genetic data base will yield homologous sequence matches to any query nucleotide sequence, additional criteria must be used to identify authentic caspase homologs from non-specific matches. Caspase family members share the highest degree of homology in the active site and catalytically important amino acid residues (Figure 2). A given EST returned by a search may not necessarily include one of these highly homologous sites but, rather, may only include a region within the

protease having cryptic homology. Confirming an EST as encoding part of a novel caspase protease involves translation of all the positive EST hits in three different reading frames and subsequent identification of conservative active site or catalytically important amino acid sequence motifs. Then, using conventional cDNA cloning, a full length cDNA of the putative novel protease can be obtained and 1) analyzed for overall structural homology to caspase family members, 2) recombinantly expressed and analyzed for cysteine protease activity, and 3) analyzed for the induction of apoptosis by heterologous expression of the cDNA in appropriate cells.

Alternative methods for isolating a caspase-14 encoding nucleic acid molecule also can be employed. For example, using the teachings described herein, those skilled in the art can routinely isolate and manipulate caspase-14 nucleic acid molecules using well known methods. All that is necessary is a disclosed sequence of a caspase-14 encoding nucleic acid molecule, for example, SEQ ID NOS:1, 4, 6, or 8, or its deduced amino acid sequence, for example, SEQ ID NOS:2, 5, 7, or 9, respectively. Such methods include, for example, screening a cDNA or genomic library by using synthetic oligonucleotides, nucleic acid fragments or primers as hybridization probes. Alternatively, antibodies to a caspase-14 polypeptide or functional fragment thereof, particularly to a caspase-14-specific epitope, can be generated and used to screen an expression library to isolate caspase-14 encoding nucleic acids.

The above described methods are known to those skilled in the art (Sambrook et al., *supra,* 1989, and the various references cited therein; Ansubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, MD, Supp. 39 (1997)). Furthermore, recombinant DNA methods currently used by those skilled in the art include the polymerase chain reaction (PCR), which, combined with the caspase-14 nucleotide and amino acid sequences described herein, allows reproduction of caspase-14 encoding sequences. Desired sequences can be amplified exponentially starting from as little as a single gene copy by means of PCR. The PCR technology is the subject matter of United States Patent Nos. 4,683,195, 4,800,159, 4,754,065, and 4,683,202.

A caspase-14 nucleic acid molecule of the invention, as well as a caspase-14 polypeptide or functional fragment thereof, can be used to diagnose, or to generate reagents to diagnose, pathological conditions associated with increased or decreased levels of apoptosis. Such methods of diagnosis include using a nucleic acid probe, which can hybridize with a caspase-14 containing nucleotide sequence, or using an antibody or ligand, which binds a caspase-14 polypeptide. Methods of diagnosis further include detecting caspase-14 enzymatic activity in a sample (see Example 2). Such methods, which are disclosed herein or otherwise known in the art, can be performed *ex vivo*, for example, by removing a cell or tissue sample from an individual exhibiting or suspected of having a pathological condition associated with increased or decreased levels of apoptosis. Correlation of increased caspase-14 expression or activity, as compared to normal levels of caspase-14, which can be determined by taking samples from apparently normal individuals, is indicative of a disease associated with increased levels of apoptosis, whereas correlation of decreased caspase-14 expression or activity is indicative of a disease associated with decreased levels of apoptosis. As used herein, reference to "increased" or "decreased" expression or activity of caspase-14 or "increased" or "decreased" levels of apoptosis means at least about one standard deviation and, preferably, at least about two standard deviations, above or below, respectively, the normal expression or activity or levels of caspase-14 in a corresponding sample of a normal individual.

A caspase-14 encoding nucleic acid of the invention, as well as a caspase-14 polypeptide or functional fragment thereof, can be used to reduce the severity of a pathological condition characterized, in part, by increased or decreased levels of apoptosis. A caspase-14 polypeptide or functional fragment thereof that includes, for example, the catalytic domain of caspase-14 can be formulated into a pharmaceutical composition and, therefore, can be used as a medicament. Such a medicament is useful in the treatment of an individual having a disease characterized, in part, by decreased levels of apoptosis, which is associated with increased cell survival and proliferation. Such a caspase-14 polypeptide or functional fragment thereof can increase the levels of apoptosis in an individual with such a disease and, thereby,

decrease cell survival and proliferation. Examples of pathological conditions associated with decreased levels of apoptosis and, therefore, increased cell survival include cancers such as lymphomas and hormone dependent tumors such as breast, prostate and ovarian cancer, autoimmune diseases such as systemic lupus erythematosus, immune-mediated glomerulonephritis and viral infections such as herpesvirus, poxvirus and adenovirus.

Additionally, molecules that interact with caspase-14, directly or indirectly, to induce caspase-14 mediated apoptosis can be used to treat such a disease. Such molecules that interact directly with caspase-14 can be identified based on their physical association with caspase-14 using, for example, an affinity matrix comprising caspase-14 or a method such as the two hybrid assay (United States Patent No. 5,283,173).

To be effective, caspase-14 polypeptides or functional fragments thereof must be introduced into cells characterized by decreased levels of apoptosis. Introduction can be accomplished by a variety of means known in the art including, for example, using lipid vesicles or receptor mediated endocytosis. Targeting the appropriate cell type also can be accomplished by conjugating the caspase-14 polypeptide or functional fragment thereof to a specific receptor ligand or a target cell specific antibody, producing a caspase-14 fusion protein comprising the ligand or antibody.

In contrast to the induction of caspase-14 mediated apoptosis for the treatment of pathological conditions characterized by increased cell survival or proliferation, inhibitors of caspase-14 can be used to treat pathological conditions associated with increased levels of apoptosis. Examples of pathological conditions associated with increased levels of apoptosis and, therefore, decreased cell survival include, for example, degenerative disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, cerebellar degeneration, myelodysplastic syndromes such as aplastic anemia and ischemic injury, including myocardial infarction, stroke and reperfusion injury.

Such inhibitors of caspase-14 can be, for example, inhibitors of the caspase-14 protease activity or inhibitors of the conversion of the inactive, proenzyme

into the active caspase-14 protease. Specific examples of such inhibitors can include, for example, anti-caspase-14 antibodies, proteins, small peptidyl protease inhibitors and small non-peptide, organic molecule inhibitors. Such inhibitors are formulated in a medium that allows introduction into the desired cell type. Alternatively, such inhibitors can be attached to targeting ligands for introduction by cell mediated endocytosis and other receptor mediated events. Specific caspase-14 peptidyl inhibitors can include suicide inhibitors and substrate analogues such as the tetrapeptide DEVD aldehyde and the cowpox virus protein Crm A, for example.

Other inhibitors of caspase-14 include, for example, small molecules or organic compounds that bind and inactivate caspase-14 by a competitive or non-competitive inhibitory type mechanism. Molecules or compounds that indirectly inhibit the caspase-14 pathway can also be used as inhibitors of caspase-14. Caspase-14 inhibitors can be identified by screening for molecules that demonstrate specific or beneficial caspase-14 inhibitory activity. Such methods are described herein and can be practiced by those skilled in the art in view of the disclosed caspase-14 nucleotide sequences and amino acid sequences.

Dominant/negative inhibitors of caspase-14 also can be used to treat or reduce the severity of pathological conditions associated with enhanced apoptosis. For example, a dominant/negative inhibitor comprising the large subunit of caspase-14, but lacking the active site QACRG (SEQ ID NO:3; positions 134-138 of SEQ ID NO:2; positions 130-134 of SEQ ID NO:5 or 7; positions 102-106 SEQ ID NO:9), can bind the small subunit of caspase-14 to form a complex that lacks protease activity. Such a mechanism of dominant negative inhibition of caspase-14 is similar to the dominant negative inhibition due to alternately spliced isoforms of caspase-2, caspase-7 and caspase-8 (Cohen, *J. Biochem. 326*:1-16 (1997)). Subunits from other caspases similarly can be used to form dominant/negative inhibitors of caspase-14 activity and, therefore, to treat pathological conditions associated with increased levels of apoptosis. Such subunits should be selected so that they bind either the large or small subunit of caspase-14 polypeptides to prevent their assembly into active heterotetrameric protease complexes. An anti-idiotypic anti-caspase-14 antibody also can serve this purpose.

Moreover, caspase-14 subunits that have been modified so as to be catalytically inactive can be used as dominant/negative inhibitors of caspase-14. Such modifications include, for example, mutation of the active site cysteine residue (amino acid position 136 of SEQ ID NO:2; amino acid position 132 of SEQ ID NO:5) to another amino acid such as alanine or glycine.

Caspase-14 substrate antagonists also can be used to treat or reduce the severity of pathological conditions associated with increased levels of apoptosis. Such substrate antagonists can bind to and inhibit cleavage by caspase-14, thereby preventing commitment progression of apoptosis. Substrate antagonists include, for example, ligands and small molecule compounds.

A caspase-14 polypeptide or functional fragment thereof, or an inhibitor of caspase-14, can be administered by conventional therapeutic methods, in dosages that are sufficient to respectively increase or decrease the levels of apoptosis in the target cells. Such dosages can be determined by those skilled in the art using, for example, Phase I and Phase II trials. Administration can be accomplished by injection, for example, intravenous, intraperitoneal or subcutaneous injection, and can be performed in a variety of different regimes, including single high dose administration, repeated small dose administration or a combination of both. The dosing will depend on the cell type, progression of the pathological condition and the overall health of the individual.

Treatment or reduction of the severity of pathological conditions associated with increased or decreased levels of apoptosis also can be accomplished by introducing expressible nucleic acid molecules encoding respectively caspase-14 polypeptides or functional fragments thereof or caspase-14 inhibitors such as antisense caspase-14 nucleic acid molecules into cells characterized by such pathological conditions. For example, treatment to reduce the severity of a pathological condition associated with decreased levels of apoptosis can be accomplished by elevating the synthesis rates of caspase-14 using recombinant caspase-14 expression vectors and gene transfer technology. Conversely, treatment or reduction of the severity of pathological conditions associated with increased levels of apoptosis can be accomplished by

introducing and expressing antisense caspase-14 nucleic acid molecules, which inhibit endogenous caspase-14 expression. Such methods of introduction and expression are well known in the art and described below with reference to recombinant viral vectors. Other vectors compatible with the appropriate targeted cell can accomplish the same goal and can be substituted in the methods described herein in place of recombinant viral vectors.

Further embodiments include the inhibition of neoplasia or apoptosis by utilizing specific antisense polynucleotides complementary to all or part of the nucleic acid sequences SEQ ID NOS:1, 4, 6, or 8 encoding a caspase-14. Such complementary antisense polynucleotides may include substitutions, additions, deletions, or transpositions, as long as specific hybridization to the relevant target sequence in SEQ ID NOS:1, 4, 6, or 8 is retained as a functional property of the polynucleotide. Antisense polynucleotides that prevent transcription and/or translation of mRNA corresponding to caspase-14 may inhibit apoptosis. Antisense polynucleotides of various lengths may be produced and used, however, the sequence length is typically at least 20 consecutive nucleotides that are substantially or wholly identical to the sequence of SEQ ID NOS:1, 3, 4, 6, or 8. (see U.S. Pat. 5,691,179 and *Antisense RNA and DNA*, D.A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 1988, each of which is incorporated herein by reference).

Recombinant viral vectors are useful for *in vivo* expression of a desired nucleic acid molecule because such vectors can offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of viral vectors such as retroviruses and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. Lateral infection can result in rapid infection of a large area, most of which were not initially infected by the original viral particles. Viral vectors that are unable to spread laterally can be useful where it is not necessary to introduce a specified gene into all of the targeted cells.

Typically, viruses infect and propagate in specific cell types. Therefore, viral vectors are useful for specifically introducing a desired gene into predetermined cell types. The vector to be used in the methods of the invention will depend on desired

cell type to be targeted. For example, if neurodegenerative diseases are to be treated by decreasing the caspase-14 activity of affected neuronal cells, then a vector specific for cells of the neuronal cell lineage, for example, herpesvirus based vectors, should be used (Kaplitt and Loewy, *Viral Vectors*, Academic Press, Inc. (1995)). Similarly, if diseases or pathological conditions of the hematopoietic system are to be treated, then a viral vector such as an HIV based vector that is specific for blood cells and their precursors, preferably for a specific type of hematopoietic cell, should be used. Moreover, such vectors can be modified with specific receptors or ligands to modify target specificity through receptor mediated events. These modification procedures can be performed by recombinant DNA techniques or synthetic chemistry procedures or the like. The specific type of vector will depend upon the intended application. Thus, the invention provides a vector that contains a nucleic acid molecule encoding a caspase-14 polypeptide or functional fragment thereof. As described herein, vectors of the invention can be used in an appropriate host cell. Thus, the invention provides a cell containing a vector of the invention. Vectors of the invention are known and readily available within the art or can be constructed by one skilled in the art using well known methodology.

A vector of the invention, such as one encoding a caspase-14 polypeptide or an inhibitor of caspase-14, for example, an antisense nucleic acid molecule, can be administered in several ways to obtain expression of such a sequence, which can increase or decrease, respectively, the level of activity of caspase-14 in the cells affected by the disease or pathological condition. If a viral vector is used, the procedure can take advantage of their target specificity and, consequently, a vector does not necessarily have to be administered locally at the diseased site. However, local administration can provide a quicker and more effective treatment. Administration can be performed by conventional methods, for example, intravenous or subcutaneous injection into the subject. Injection of a viral vector into the spinal fluid also can be used as a mode of administration, especially in the case of neurodegenerative diseases of the central nervous system. Following injection, the viral vector will bind to a target cell expressing an appropriate receptor.

A caspase-14 encoding vector can be administered locally at the site of the disease or pathological condition. Local administration is advantageous because there is no dilution effect and, therefore, a smaller dose is required to achieve caspase-14 expression in a majority of the targeted cells. Additionally, local administration can alleviate the targeting requirement of other forms of administration, since a vector can be used that infects all cells in the locally administered area. If expression is desired in only a specific subset of cells within the administered area, then promoter and expression elements that are specific for the desired subset can be incorporated in the vector, which can be a viral vector, viral genome, plasmid or phagemid. A transfection vehicle such as a liposome can be used to introduce the vector into recipient cells within the inoculated area. Such transfection vehicles are known by those skilled in the art. Alternatively, the vector can be administered directly into a tissue of an individual (Wolff et al., *Science 247*:1465-1468 (1990)).

Additional features can be added to a vector to ensure safety and/or enhance therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene that confers sensitivity to the antibiotic gancyclovir. Negative selection is a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic.

Additionally, a caspase-14 encoding nucleic acid molecule of the invention, as well as a caspase-14 polypeptide or functional fragment thereof, can be used to screen for pharmaceutical compounds and macromolecules that modulate, that is, inhibit or enhance, caspase-14 activity. Such a caspase-14 encoding nucleic acid molecule, caspase-14 polypeptide or functional fragment thereof can be used in a sample to screen for inhibitors of caspase-14, including those that inhibit enzymatic or apoptotic activity. Alternatively, a caspase-14 encoding nucleic acid molecule, caspase-14 polypeptide or functional fragment thereof can be used in a sample to screen for compounds that enhance caspase-14 activity such as by inducing cleavage of the caspase-14 proenzyme into its active subunits. Such a sample can contain a cell lysate

and also can contain isolated caspase-14 encoding nucleic acid molecules, caspase-14 polypeptides or functional fragments thereof.

Candidate inhibitors and enhancers may be isolated or procured from a variety of sources, such as bacteria, fungi, plants, parasites, libraries of chemicals, peptides or peptide derivatives and the like. Inhibitors and enhancers may be also be rationally designed, based on the protein structure determined from X-ray crystallography (see, Mittl et al., *J. Biol. Chem.*, *272*:6539-6547, 1997). In certain preferred embodiments, the inhibitor targets a specific caspase (*e.g.*, caspase-3 and not any other caspases).

Without being held to a particular mechanism, the inhibitor may act by preventing processing of caspase or by preventing enzymatic activity, or by other mechanism. The inhibitor may act directly or indirectly. In preferred embodiments, inhibitors interfere in the processing of the caspase protein. In other preferred embodiments, the inhibitors are small molecules. In a most preferred embodiment, the inhibitors prevent apoptosis. Inhibitors should have a minimum of side effects and are preferably non-toxic. Inhibitors that can penetrate cells are preferred.

In addition, enhancers of caspase activity or expression are desirable in certain circumstances. At times, increasing apoptosis will have a therapeutic effect. For example, tumors or cells that mediate autoimmune diseases are appropriate cells for destruction. Enhancers may increase the rate or efficiency of caspase processing, increase transcription or translation, or act through other mechanisms. As is apparent to one skilled in the art, many of the guidelines presented above apply to the design of enhancers as well.

Screening assays for inhibitors and enhancers will vary according to the type of inhibitor or enhancer and the nature of the activity that is being affected. Assays may be performed *in vitro* or *in vivo*. In general, *in vitro* assays are designed to evaluate caspase protein processing or caspase enzymatic activity, and *in vivo* assays are designed to evaluate caspase protein processing, caspase enzymatic activity, apoptosis, or caspase cleavage of substrate. In any of the assays, a statistically significant increase or decrease compared to a proper control is indicative of enhancement or inhibition.

One type of *in vitro* assay can be performed by examining the effect of a candidate compound on the processing of caspase-14 into two subunits. Briefly, a caspase-14, that is a primary translation product, is obtained from an *in vitro* translation system. The caspase-14 is preferably constructed to be capable of normal auto-processing, but can be constructed to be cleaved by other protease components present or added to the reaction. This primary product is contacted with or without, or translated in the presence or absence of a candidate compound and assessed for appearance of the two subunits. To facilitate detection, typically, the caspase-14 is labeled during translation. The two subunits may be readily detected by autoradiography after gel electrophoresis. One skilled in the art will recognize that other methods of labeling and detection may be used alternatively.

An alternative *in vitro* assay is designed to measure cleavage of a caspase substrate (*e.g.*, Acetyl DEVD-aminomethyl coumarin (amc), lamin, PRPP, and the like). Substrate turnover may be assayed using either cleavable or noncleavable rev-caspase. Briefly, in this method, caspase-14 is translated and allowed sufficient time to be processed or subjected to a protease which activates caspase-14. The caspase substrate along with the candidate compound is added to the reaction. Detection of cleaved substrate is performed by any one of a variety of standard methods. Generally, the substrate will be labeled and followed by an appropriate detection means.

Moreover, any known enzymatic analysis can be used to follow the inhibitory or enhancing ability of a candidate compound with regard to a caspase-14 of this invention. For example, one could express caspase-14 in a cell line be it bacterial, insect, mammalian or other, and purify the caspase. The purified caspase-14 could then be used in a variety of assays to follow its catalytic ability in the presence of candidate compounds, as noted above. Such methods of expressing and purifying recombinant proteins are known in the art and examples can be found in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, 1989 as well as in a number of other sources.

In vivo assays are typically performed in cells transfected either transiently or stably with an expression vector containing a caspase-14 gene, such as

those described herein. These cells are used to measure caspase-14 processing, substrate turnover, or apoptosis in the presence or absence of a candidate compound. When assaying apoptosis, a variety of cell analyses may be used including, for example, dye staining and microscopy to examine nucleic acid fragmentation and porosity of the cells. Further, *in vivo* assaying for the ability of the transfected caspase-14 to cleave known substrates that are co-transfected or placed in the cell culture media in the presence of the candidate compound can be performed thereby allowing for the detection and determination of substrate turnover.

The assays briefly described herein may be used to identify an enhance or inhibitor that is specific for an individual caspase. In a preferred embodiment candidate compounds would be analyzed using a variety of caspases (*e.g.*, caspase-1 through caspase-14) to identify specific inhibitors and enhancers for individual caspases.

A variety of methodologies exist can be used to investigate the effect of a candidate compound. Such methodologies are those commonly used to analyze enzymatic reactions and include, for example, SDS-PAGE, spectroscopy, HPLC analysis, autoradiography, chemiluminescence, chromogenic reactions, and immunochemistry (*e.g.*, blotting, precipitating, etc.).

Inhibitors and enhancers may be used in the context of this invention to exert control over the cell death process or cytokine activation. Thus, these inhibitors and enhancers will have utility in diseases characterized by either excessive or insufficient levels of apoptosis. Inhibitors of caspase proteases have potential to treat the major neurodegenerative diseases: stroke, Parkinson's Disease, Alzheimer's Disease, and ALS. As well, caspase-14 protease inhibitors may be used to inhibit apoptosis in the heart following myocardial infarction, in the kidney following acute ischemia, and in diseases of the liver. Enhancers of caspase-14 activity may be used in contexts when apoptosis or cytokine activation are desired. For example, inducing or increasing apoptosis in cancer cells or aberrantly proliferating cells may be effected by delivery of a caspase enhancer.

Such screening methods are known to those skilled in the art and can be performed by either *in vitro* or *in vivo* procedures. For example, Example 2 provides a specific *in vitro* assay for caspase-14 protease activity. This assay employs a sample containing a caspase-14 polypeptide expressed in an active, processed form recombinantly in *E. coli*. The protease activity of the polypeptide is measured by incubation with a fluorescent substrate. This assay can be used to screen synthetic or naturally occurring compound libraries, including macromolecules, for agents that either inhibit or enhance caspase-14 activity. The caspase-14 polypeptides or functional fragments thereof to be used in the assay can be obtained by, for example, *in vitro* translation, recombinant expression or biochemical procedures. Methods other than that described in Example 2 also can be used to screen and identify compounds that inhibit or enhance caspase-14 activity including, for example, those described *supra* and other methodologies such as using phage display peptide libraries, where greater than 10^8 peptide sequences can be screened in a single round of panning. Such methods, as well as others, are known in the art and can be utilized to identify compounds that inhibit or enhance caspase-14 activity.

As noted above, caspase-14 nucleic acid molecules may be delivered to cells in combination with a vector or other gene delivery vehicle. These methods may be accomplished by delivery of DNA or cDNA capable of *in vivo* transcription caspase-14 or an active fragment thereof. More specifically, in order to produce caspase-14 *in vivo*, a nucleic acid sequence coding for caspase-14 is placed under the control of a eukaryotic promoter (*e.g.*, a pol III promoter, CMV or SV40 promoter). Where it is desired to more specifically control transcription, the caspase-14 encoding nucleic acid molecule may be placed under the control of a tissue or cell specific promoter (*e.g.*, to target cells in the liver), or an inducible promoter, such as MMTV LTR, CMV IE promoter, RSV LTR, SV40, metallothionein promoter (*see, e.g.*, U.S. Patent No. 4,870,009), ecdysone response element system, tetracycline-reversible silencing system (tet-on, tet-off), and the like.

Many techniques for introduction of nucleic acids into cells are known. Such methods include retroviral vectors and subsequent retrovirus infection, adenoviral

or adeno-associated viral vectors and subsequent infection, and complexes of nucleic acid with a condensing agent (*e.g.*, poly-lysine). These complexes or viral vectors may be targeted to particular cell types by way of a ligand incorporated into the vehicle. Many ligands specific for tumor cells and other cells are well known in the art.

A wide variety of vectors may be utilized within the context of the present invention, including for example, plasmids, viruses, retrotransposons and cosmids. Representative examples include adenoviral vectors (*e.g.*, WO 94/26914, WO 93/9191; Yei *et al.*, *Gene Therapy 1*:192-200, 1994; Kolls *et al.*, *PNAS 91*(1):215-219, 1994; Kass-Eisler *et al.*, *PNAS 90*(24):11498-502, 1993; Guzman *et al.*, *Circulation 88*(6):2838-48, 1993; Guzman *et al.*, *Cir. Res. 73*(6):1202-1207, 1993; Zabner *et al.*, *Cell 75*(2):207-216, 1993; Li *et al.*, *Hum Gene Ther. 4*(4):403-409, 1993; Caillaud *et al.*, *Eur. J. Neurosci. 5*(10):1287-1291, 1993), adeno-associated type 1 ("AAV-1") or adeno-associated type 2 ("AAV-2") vectors (*see* WO 95/13365; Flotte *et al.*, *PNAS 90*(22):10613-10617, 1993), hepatitis delta vectors, live, attenuated delta viruses and herpes viral vectors (*e.g.*, U.S. Patent No. 5,288,641), as well as vectors which are disclosed within U.S. Patent No. 5,166,320. Other representative vectors include retroviral vectors (*e.g.*, EP 0 415 731; WO 90/07936; WO 91/02805; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 93/11230; WO 93/10218.

Within certain aspects of the invention, nucleic acid molecules that encode caspase-14 may be introduced into a host cell utilizing a gene delivery vehicle, or by various physical methods. Representative examples of such methods include transformation using calcium phosphate precipitation (Dubensky *et al.*, *PNAS 81*:7529-7533, 1984), direct microinjection of such nucleic acid molecules into intact target cells (Acsadi *et al.*, *Nature 352*:815-818, 1991), and electroporation whereby cells suspended in a conducting solution are subjected to an intense electric field in order to transiently polarize the membrane, allowing entry of the nucleic acid molecules. Other procedures include the use of nucleic acid molecules linked to an inactive adenovirus (Cotton *et al.*, *PNAS 89*:6094, 1990), lipofection (Felgner *et al.*, *Proc. Natl. Acad. Sci. USA 84*:7413-7417, 1989), microprojectile bombardment (Williams *et al.*, *PNAS 88*:2726-2730,

1991), polycation compounds such as polylysine, receptor specific ligands, liposomes entrapping the nucleic acid molecules, spheroplast fusion whereby *E. coli* containing the nucleic acid molecules are stripped of their outer cell walls and fused to animal cells using polyethylene glycol, viral transduction, (Cline *et al.*, *Pharmac. Ther. 29*:69, 1985; and Friedmann *et al.*, *Science 244*:1275, 1989), and DNA ligand (Wu *et al.*, *J. Biol. Chem. 264*:16985-16987, 1989), as well as psoralen inactivated viruses such as Sendai or Adenovirus. In one embodiment, the caspase-14 encoding construct is introduced into the host cell using a liposome.

In an additional embodiment, the compositions of caspase-14 may be administered either alone, or as a pharmaceutical composition. These compositions may contain any of the above described inhibitors, enhancers, DNA molecules, vectors or host cells, along with a pharmaceutically or physiologically acceptable carrier, excipients or diluents. Generally, such carriers should be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the therapeutic agent with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents.

Compositions of the present invention may be formulated for the manner of administration indicated, including for example, for oral, nasal, venous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration. Within other embodiments of the invention, the compositions described herein may be administered as part of a sustained release implant. Within yet other embodiments, compositions of the present invention may be formulized as a lyophilizate, utilizing appropriate excipients which provide stability as a lyophilizate, and subsequent to rehydration. One skilled in the art may further formulate the enhancers or inhibitors of this invention in an appropriate manner, and in accordance with accepted practices, such as those disclosed in *Remington's Pharmaceutical Sciences*, Gennaro, Ed., Mack Publishing Co.,

Easton, PA 1990. Pharmaceutical compositions are useful for both diagnostic or therapeutic purposes.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease. Dosages may be determined most accurately during clinical trials. Patients may be monitored for therapeutic effectiveness by appropriate technology, including signs of clinical exacerbation, imaging and the like.

It is understood that modifications that do not substantially affect the various embodiments of the invention also are included within the invention. Accordingly, the following examples are intended to illustrate but not limit the present invention.

EXAMPLES

EXAMPLE 1

CHARACTERIZATION OF CASPASE-14

This example shows the sequencing and analysis of caspase-14.

An EST, GenBank accession number AA103647, was identified during a homology search of the GenBank database using a query nucleotide sequence based on caspase-3 and caspase-6 coding sequences. The EST is a sequence of 483 nucleotides in length.

The EST was derived from a mouse cell clone, which was obtained from IMAGE Consortium. The EST was resequenced, revealing that it contained numerous sequencing errors, including at nucleotide positions 13, 54 and 164 of SEQ ID NO:1, where the corresponding positions in the EST contain nothing. Full sequencing of the clone revealed a sequence of 850 nucleotides in length encoding a polypeptide (SEQ ID NO:2), designated herein as caspase-14, which is similar to members of the caspase family of proteases.

Following this characterization, amplification of a partial human caspase-14 cDNA from a human brain cDNA library was conducted. The set of forward and reverse primers derived from the mouse cDNA sequence of caspase-14 are set forth below:

Forward primer: ATATGATATGTCAGGTGCCCG (SEQ ID NO:__)

Reverse primer: TTCCGGAGGGTGCTTTGGA (SEQ ID NO:__)

To obtain the 5' and 3' coding sequences of human caspase-14 we performed RACE (rapid amplification of cDNA ends) using nested PCR primers derived from the human caspase-14 cDNA and vector specific primers complimentary to the library vector.

For 5' amplification

Reverse primers: CCTGTATGATGTACACCTTGG (SEQ ID NO:__)

AGAGATTCTCCAGCTTGAC (SEQ ID NO:__)
ATCTTCTCCCTTGAGGAAG (SEQ ID NO:__)

For 3' amplification
Forward primers: ATATGATATGTCAGGTGCCCG (SEQ ID NO:__)
CAAGGTGTACATCATACAGG (SEQ ID NO:__)

Following the above PCR amplification, the derived sequence (SEQ ID NO:4) and the predicted amino acid sequence (SEQ ID NO:5) were compared with the mouse sequence. The human and mouse proteins exhibit 75% identity when compared using the GCG pileUp program (Gapweight:12, Gaplength weight:4). The pileUp program creates a multiple sequence alignment form a group of related sequences using progressive, pairwise alignments. PileUp creates a multiple sequence alignment using the progressive alignment method of Feng and Doolittle (*J. Mol. Evol. 25*:351-360, 1987) and is similar to the method described by Higgins and Sharp (*CABIOS 5*:151-153, 1989).

EXAMPLE 2

KINETIC PARAMETERS OF CASPASE-14

This example provides methods to characterize the protease activity and substrate specificity of caspase-14.

The kinetic properties of bacterially expressed recombinant caspase-14 is determined using tetrapeptide substrates in a continuous fluorometric assay. Examples of two such substrates are DEVD-AMC and the YVAD-AMC, which represent the cleavage sites for the poly(ADP-ribose)polymerase (PARP) and IL-1β P1-P4 substrate tetrapeptides, respectively (Nicholson et al., *Nature 376*:37-43 (1995)). Caspase-14 cDNA lacking most of the propeptide coding sequence is subcloned in-frame into the *Bam HI/Xho1* sites of the bacterial expression vector pGEX-5X-3 (Pharmacia Biotech Inc.). This vector produces caspase-14 as a fusion protein with glutathione S-transferase (GST) and is used essentially as described in Fernandes-Alnemri et al., *Cancer Res. 55*:6045-6052 (1995). The GST-caspase-14 expression vector is

constructed and transformed into DH5α bacteria using routine molecular biology methods known to those skilled in the art. After induction with IPTG, bacterial extracts are prepared from *E. coli* expressing the recombinant fusion proteins. The extracts are adsorbed to glutathione-Sepharose resin, washed several times and then analyzed by SDS-PAGE.

The isolated caspase-14 GST-fusion protein is then used for further enzymatic analyses. The activity of caspase-14 is measured using bacterial lysates prepared with ICE buffer (25 mM HEPES, 1 mM EDTA, 5mM DTT, 0.1% CHAPS, 10% sucrose, pH 7.5) at room temperature (24-25°C). The K_i's are determined from the hydrolysis rate of 50 μM DEVD-AMC following a 30 min preincubation of the enzyme with inhibitors DEVD-CHO and recombinant CrmA protein. Prior to incubation with enzyme, purified CrmA is activated by incubation with 5 mM DTT for 10 min at 37°C.

Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention.

EXAMPLE 3

OVEREXPRESSION OF PROCASPASE-14 IN MCF-7 CELLS

Overexpression of small prodomain executioner procaspases such as procaspase-3 or -6 in mammalian cells does not induce apoptosis, due to their inability to autoprocess themselves. On the other hand overexpression of the large prodomain initiator procaspases such as procaspase-8 can induce apoptosis, due to their ability to undergo prodomain-mediated oligomerization. To test the ability of procaspase-14 to induce apoptosis in transfected cells, MCF-7 cells were transiently transfected with procaspase-14 in a PRSC lac-Z expression construct under the CMV promoter. Cells were also transfected with an empty vector or constructs encoding procaspase -3, -6, -8, or -10 as controls. The cells were stained with X-gal 30 h after transfection and examined for morphological signs of apoptosis. The percentage of round blue apoptotic

cells (mean ± SD) were represented as a function of total blue cells under each condition ($n \geq 3$). SD was less than 5%. As depicted in Figure 4, overexpression of procaspase-3 as well as procaspase-14, but not procaspase-8 or -10, was unable to induce any significant amount of apoptosis. This suggests that procaspase-14, like other executioner procaspases with small prodomains, can not undergo self activation to induce apoptosis.

EXAMPLE 4

EXPRESSION OF PROCASPASE-14

Overexpression of procaspase-14 and procaspase-3 in bacteria. Procaspase-14 and procaspase-3 were expressed in *Escherichia coli* purified on Talon Ni^{+2}-affinity resin (Clontech, and then analyzed by SDS-PAGE and Coomassie staining. As depicted in Figure 5A, *Lane M*, molecular mass markers (kDa); *lane casp-14*, Talon-affinity purified caspase-14; *lane casp-3*, Talon-affinity purified caspase-3.

EXAMPLE 5

PROCESSING OF PROCASPASE-14

Upon expression in bacteria, all procaspases are known to autoprocess to various degrees to generate the mature caspase which is composed of the large and small subunits. The observed auto-activation in bacterial overexpression systems is probably mediated by overexpression-induced oligomerization. Oligomerization has been shown to induce autoactivation/processing of procaspases. Interestingly, when procaspase-14 was overexpressed in bacteria there was no significant processing of its proenzyme compared to procaspase-3 which was completely processes (Figure 5A). This suggests that procaspase-14 does not normally process itself and it may require an upstream protease to process it. To test this possibility, procaspase-14 was incubated with Granzyme B which is known to process several caspase proenzymes. In addition procaspase-14 was incubated with different purified recombinant caspases. As shown in Figure 5B, a significant amount of processing was observed when procaspase-14 was

incubated with Granzyme B, caspase-10 and caspase-8, but not with other caspases. Some processing was also observed with recombinant caspase-14 itself, indicating that the purified caspase-14 material contains small amount of active capase-14. These observations suggest that procaspase-14 may participate in the Granzyme B, caspase-8 and caspase-10 protease cascades.

Processing of mouse procaspase-14 by Granzyme B and purified recombinant caspases was carried out under the following conditions: ^{35}S labeled procaspase-14 was incubated with purified Granzyme B (14 ng/μl) or the indicated purified recombinant caspases (20 ng/μl) in ICE buffer (25 mM Hepes, 1 mM EDTA, 5mM DTT, 0.1% CHAPS, pH 7.5) at 37° C for 1h. The reactions were stopped by addition of an SDS-sample buffer and then the products were analyzed by SDS-PAGE and autoradiography.

Since caspase-8 and -10 are initiator caspases that are activated by oligomerization of the death receptors (*i.e.*, Fas, TRAIL-R) by their ligands or agonist antibodies, the possibility that procaspase-14 is processed *in vivo* after induction of apoptosis by anti-Fas antibody or the cytotoxic ligand TRAIL was tested. A mammalian expression construct encoding N-terminal T7-tagged procaspase-14 was transfected into MCF-7-FAS cells. The cells were treated 36 h after transfection with agonist anti-Fas antibody or TRAIL for 3 h. Cells were harvested and lysed by addition of SDS-sample buffer. The cellular proteins were analyzed by SDS-PAGE and then immunoblotted with an anti-T7 HRP-conjugated monoclonal antibody (Figure 5C, *left panel*) to detect procaspase-14. The same samples were also immunoblotted with a polyclonal antibody (anti-Mch3α) that preferentially detects the proform of caspase-7 (Figure 5C, *middle panel*), or a mixture of the anti-Mch3α antibody and CM-1 antibody that preferentially detects the processes fragments of caspase-7 (Figure 5C, *right panel*) to detect the endogenous caspase-7. Pro indicates the proenzyme, LS indicates the large subunit and SS indicates the small subunit. As shown in Figure 5C, Anti-Fas and TRAIL were able to induce processing of procaspase-14 and procaspase-7 as evident from the decreased intensity of their proenzyme bands. The cleavage products of procaspase-14 were not clearly detectable probably due to loss of the epitope tag after

processing of the small prodomain of procaspase-14 at Asp7 or Asp17. On the other hand, the cleavage products of procaspase-7 were clearly detectable using an antibody that detects the processes large subunit of procaspase-7 (Figure 5C, right panel). These observations indicate that caspase-14, like caspase-7, is likely involved in the death receptor pathways.

EXAMPLE 6

CYTOCHROME C DEPENDENT PROCESSING OF PROCASPASE-14

To determine if caspase-14 was activated by a way of Apaf-1 and caspase-9, cytochrome c dependent activation was tested. ^{35}S labeled procaspase-3 (Figure 6, *lanes 1 & 2*), procaspase-7 (Figure 6, *lanes 3 & 4*), or procaspase-14 (Figure 6, *lanes 5 & 6*) were incubated with S100 extracts from human embryonic kidney 293 cells in the absence (-) or presence (+) of cytochrome c (50 ng/μl) and dATP (1mM) in buffer A (20 mM Hepes, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.1 mM PMSF, pH 7.5) at 30°C for 1 h. The reaction products were then analyzed by SDS-PAGE and autoradiography.

As indicated by Figure 6, unlike caspase-3 or -7, no cleavage/activation of ^{35}S labeled procaspase-14 was observed in S-100 extracts activated by cytochrome c and dATP. This indicates that cytochrome c-activated caspase-3, -6, -7, and -9 in the S-100 cellular extract likely cannot process procaspase-14.

CLAIMS

We claim:

1. An isolated nucleic acid molecule encoding a caspase-14 polypeptide or functional fragment thereof.

2. The isolated nucleic acid molecule of claim 1, comprising a contiguous single stranded or double stranded nucleotide sequence of SEQ ID NO:4.

3. The isolated nucleic acid molecule of claim 2, wherein said single stranded nucleotide sequence comprises a contiguous nucleotide sequence of coding strand or non-coding strand.

4. The isolated nucleic acid molecule of claim 1, which encodes the amino acid sequence of SEQ ID NO:5 or a splice variant thereof.

5. An isolated nucleic acid molecule, comprising at least 12 contiguous nucleotides of the nucleic acid molecule of claim 1 or SEQ ID NO:4, provided that said contiguous nucleotides do not consist entirely of positions 430 to 450 of SEQ ID NO:4, positions 436 to 453 of SEQ ID NO:1, the expressed sequence tag having GenBank accession number AA103647, or any contiguous portion thereof.

6. An isolated nucleic acid molecule, comprising a caspase-14 gene or functional fragment thereof.

7. The isolated nucleic acid molecule of claim 6, wherein said functional fragment is a regulatory element.

8. A vector, comprising the nucleic acid molecule of claim 1.

9. The vector of claim 8, which is a viral vector.

10. An expression vector, comprising the nucleic acid molecule of any one of claims 1-7, wherein the nucleic acid molecule is operatively linked to a promoter.

11. A host cell containing the vector of claim 8.

12. An isolated caspase-14 polypeptide or functional fragment thereof.

13. The isolated polypeptide of claim 12, comprising SEQ ID NO:5 or a splice variant thereof.

14. The polypeptide of claim 12, comprising a functional fragment of SEQ ID NO:5 or a splice variant thereof.

15. The polypeptide of claim 12, wherein said functional fragment comprises a large subunit of caspase-14.

16. The polypeptide of claim 12, wherein said functional fragment comprises a small subunit of caspase-14.

17. The polypeptide of claim 14, wherein said functional fragment comprises contiguous amino acid sequences selected from the group consisting of positions 1 to 146 of SEQ ID NO:5, positions 1-146 of SEQ ID NO:7, and positions 1-118 of SEQ ID NO:9.

18. The polypeptide of claim 14, wherein said functional fragment comprises contiguous amino acid sequences selected from the group consisting of positions 147 to 242 of SEQ ID NO:5, positions 147-230 of SEQ ID NO:7, and positions 119-214 of SEQ ID NO:9.

19. The polypeptide of claim 15, wherein said large subunit is fused to a small subunit of a polypeptide selected from the group consisting of caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10 and ced-3.

20. The polypeptide of claim 16, wherein said small subunit is fused to a large subunit of a polypeptide selected from the group consisting of caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10 and ced-3.

21. An anti-caspase-14 antibody.

22. The antibody of claim 21, which is a monoclonal antibody.

23. A cell expressing the antibody of claim 22.

24. An anti-idiotypic anti-caspase-14 antibody.

25. A method for identifying a compound that modulates caspase-14 activity, comprising the steps of:

a) contacting a sample containing a caspase-14 polypeptide or functional fragment thereof with a test compound; and

b) determining the activity of said caspase-14 polypeptide or functional fragment thereof, wherein a change in activity indicates a compound that modulates caspase-14 activity.

26. The method of claim 25, wherein said compound inhibits caspase-14 activity.

27. The method of claim 25, wherein said compound enhances caspase-14 activity.

28. The method of claim 25, wherein said caspase-14 activity is apoptotic activity.

29. The method of claim 25, wherein said caspase-14 activity is determined by a binding assay.

30. The method of claim 25, wherein said caspase-14 activity is enzymatic activity.

31. The method of claim 30, wherein said enzymatic activity is determined by contacting said caspase-14 polypeptide or functional fragment thereof with a fluorescent substrate.

32. The method of claim 31, wherein said fluorescent substrate is selected from the group consisting of DEVD-AMC and YVAD-AMC.

33. The method of claim 25, wherein said sample comprises a cell lysate.

34. The method of claim 25, wherein said sample comprises an isolated caspase-14 polypeptide or functional fragment thereof.

35. The method of claim 25, wherein said compound is a small molecule.

36. The method of claim 25, wherein said compound is an anti-caspase-14 antibody.

37. The method of claim 25, wherein said compound is an anti-idiotypic anti-caspase-14 antibody.

38. A method of identifying inhibitors or enhancers of caspase-14 activity comprising:

a) contacting an activated caspase-14 with a substrate in the presence of a test compound under conditions in which the caspase-14 processes the substrate in the absence of the test compound; and thereafter

b) detecting increased or decreased substrate turnover, wherein increased substrate turnover indicates the presence of an enhancer and wherein decreased substrate turnover indicates the presence of an inhibitor.

39. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO:6.

40. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO:8.

41. The isolated polypeptide of claim 12, comprising the polypeptide sequence of SEQ ID NO:7.

42. The isolated polypeptide of claim 12, comprising the polypeptide sequence of SEQ ID NO:9.

43. An isolated nucleic acid molecule encoding a human caspase-14 polypeptide.

44. An isolated human caspase-14 polypeptide.

45. The isolated nucleic acid molecule of claim 1, which comprises a contiguous single stranded or double stranded nucleotide sequence of SEQ ID NO:1.

46. The isolated nucleic acid molecule of claim 2, wherein said single stranded nucleotide sequence comprises a contiguous nucleotide sequence of coding strand or non-coding strand.

47. The isolated nucleic acid molecule of claim 1, which encodes the amino acid sequence of SEQ ID NO:2.

48. An isolated nucleic acid molecule, comprising at least 12 contiguous nucleotides of the nucleic acid molecule of claim 1 or SEQ ID NO:1, provided that said contiguous nucleotides do not consist of positions 454 to 474 of SEQ ID NO:1, positions 460 to 477 of SEQ ID NO:1, the expressed sequence tag having GenBank accession number AA103647, or any contiguous portion thereof.

49. An isolated nucleic acid molecule, comprising a caspase-14 gene or functional fragment thereof.

50. The isolated nucleic acid molecule of claim 6, wherein said functional fragment is a regulatory element.

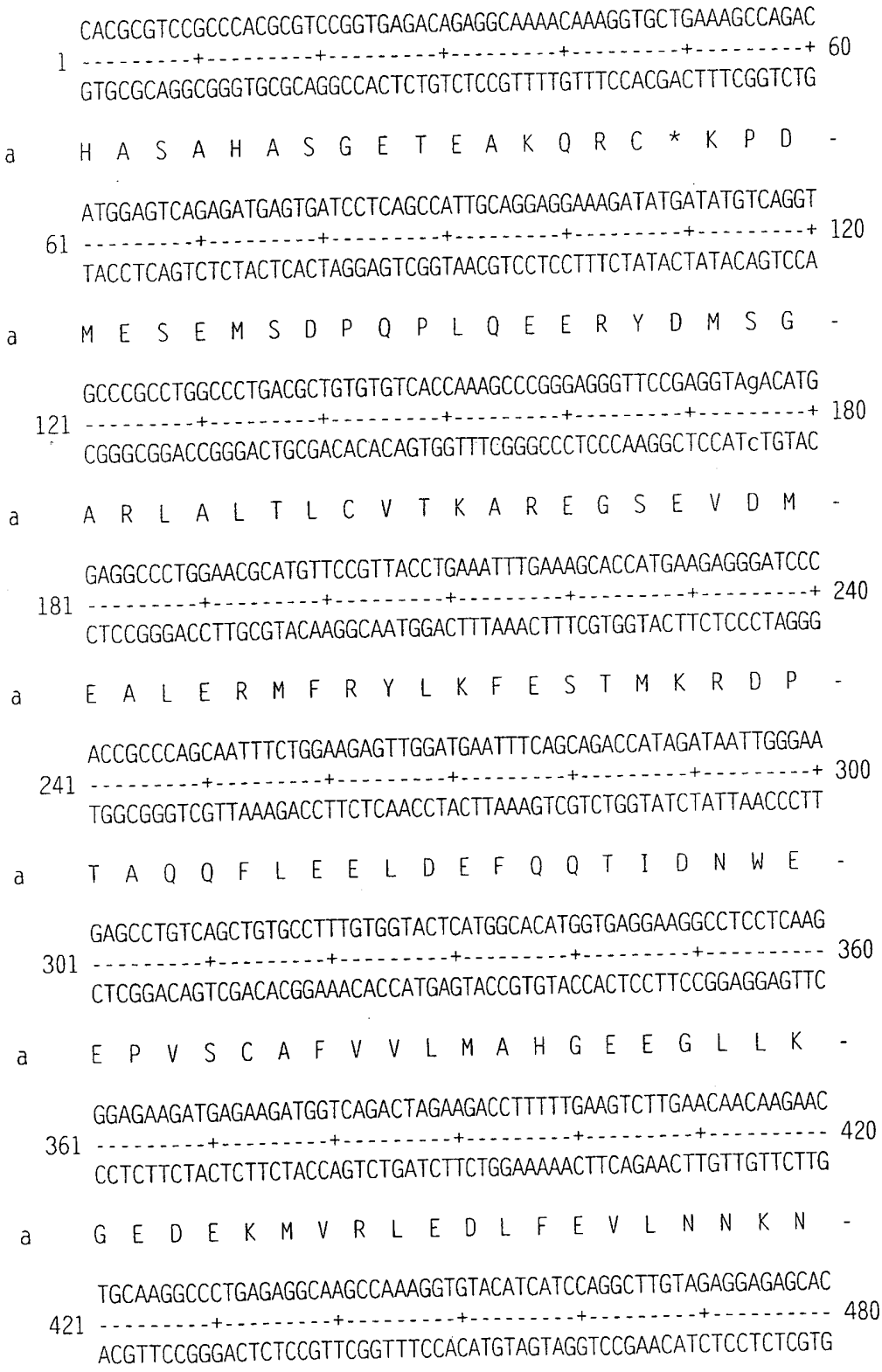
51. An expression vector, comprising the nucleic acid molecule of any one of claims 45-50, wherein the nucleic acid molecule is operatively linked to a promoter.

52. The isolated polypeptide of claim 12. comprising SEQ ID NO:2.

53. The polypeptide of claim 12, comprising a functional fragment of SEQ ID NO:2.

54. The polypeptide of claim 53, wherein said functional fragment comprises positions 1 to 156 of SEQ ID NO:2.

55. The polypeptide of claim 53, wherein said functional fragment comprises positions 163 to 257 of SEQ ID NO:2.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1 --------+--------+--------+--------+--------+--------+ 60
GTGCGCAGGCGGGTGCGCAGGCCACTCTGTCTCCGTTTTGTTTCCACGACTTTCGGTCTG
a H A S A H A S G E T E A K Q R C * K P D -
ATGGAGTCAGAGATGAGTGATCCTCAGCCATTGCAGGAGGAAAGATATGATATGTCAGGT
61 --------+--------+--------+--------+--------+--------+ 120
TACCTCAGTCTCTACTCACTAGGAGTCGGTAACGTCCTCCTTTCTATACTATACAGTCCA
a M E S E M S D P Q P L Q E E R Y D M S G -
GCCCGCCTGGCCCTGACGCTGTGTGTCACCAAAGCCCGGGAGGGTTCCGAGGTAgACATG
121 --------+--------+--------+--------+--------+--------+ 180
CGGGCGGACCGGGACTGCGACACACAGTGGTTTCGGGCCCTCCCAAGGCTCCATcTGTAC
a A R L A L T L C V T K A R E G S E V D M -
GAGGCCCTGGAACGCATGTTCCGTTACCTGAAATTTGAAAGCACCATGAAGAGGGATCCC
181 --------+--------+--------+--------+--------+--------+ 240
CTCCGGGACCTTGCGTACAAGGCAATGGACTTTAAACTTTCGTGGTACTTCTCCCTAGGG
a E A L E R M F R Y L K F E S T M K R D P -
ACCGCCCAGCAATTTCTGGAAGAGTTGGATGAATTTCAGCAGACCATAGATAATTGGGAA
241 --------+--------+--------+--------+--------+--------+ 300
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a T A Q Q F L E E L D E F Q Q T I D N W E -
GAGCCTGTCAGCTGTGCCTTTGTGGTACTCATGGCACATGGTGAGGAAGGCCTCCTCAAG
301 --------+--------+--------+--------+--------+--------+ 360
CTCGGACAGTCGACACGGAAACACCATGAGTACCGTGTACCACTCCTTCCGGAGGAGTTC
a E P V S C A F V V L M A H G E E G L L K -
GGAGAAGATGAGAAGATGGTCAGACTAGAAGACCTTTTTGAAGTCTTGAACAACAAGAAC
361 --------+--------+--------+--------+--------+--------+ 420
CCTCTTCTACTCTTCTACCAGTCTGATCTTCTGGAAAAACTTCAGAACTTGTTGTTCTTG
a G E D E K M V R L E D L F E V L N N K N -
TGCAAGGCCCTGAGAGGCAAGCCAAAGGTGTACATCATCCAGGCTTGTAGAGGAGAGCAC
421 --------+--------+--------+--------+--------+--------+ 480
ACGTTCCGGGACTCTCCGTTCGGTTTCCACATGTAGTAGGTCCGAACATCTCCTCTCGTG

Fig. 1A

```
a    C  K  A  L  R  G  K  P  K  V  Y  I  I  Q  A  C  R  G  E  H  -

     AGAGACCCCGGTGAGGAACTACGTGGAAATGAGGAACTAGGTGGAGATGAGGAACTNGGT
 481 ---------+---------+---------+---------+---------+---------+ 540
     TCTCTGGGGCCACTCCTTGATGCACCTTTACTCCTTGATCCACCTCTACTCCTTGANCCA

a    R  D  P  G  E  E  L  R  G  N  E  E  L  G  G  D  E  E  L  G  -

     GGAGATGAGGTTGCTGTGCTCAAGAACAACCCCCAAAGTATCCCAACCTATACGGATACC
 541 ---------+---------+---------+---------+---------+---------+ 600
     CCTCTACTCCAACGACACGAGTTCTTGTTGGGGGTTTCATAGGGTTGGATATGCCTATGG

a    G  D  E  V  A  V  L  K  N  N  P  Q  S  I  P  T  Y  T  D  T  -

     CTCCACATCTACTCCACGGTAGAGGGGTACCTCTCCTATAGACATGACGAGAAAGGCTCT
 601 ---------+---------+---------+---------+---------+---------+ 660
     GAGGTGTAGATGAGGTGCCATCTCCCCATGGAGAGGATATCTGTACTGCTCTTTCCGAGA

a     L  H  I  Y  S  T  V  E  G  Y  L  S  Y  R  H  D  E  K  G  S  -

     GGCTTCATCCAGACCCTGACGGATGTGTTCATTCATAAAAAAGGATCCATCTTAGAACTG
 661 ---------+---------+---------+---------+---------+---------+ 720
     CCGAAGTAGGTCTGGGACTGCCTACACAAGTAAGTATTTTTTCCTAGGTAGAATCTTGAC

a    G  F  I  Q  T  L  T  D  V  F  I  H  K  K  G  S  I  L  E  L  -

     ACAGAAGAGATCACCCGACTTATGGCAAACACGGAGGTGATGCAGGAAGGAAAACCAAGG
 721 ---------+---------+---------+---------+---------+---------+ 780
     TGTCTTCTCTAGTGGGCTGAATACCGTTTGTGCCTCCACTACGTCCTTCCTTTTGGTTCC

a    T  E  E  I  T  R  L  M  A  N  T  E  V  M  Q  E  G  K  P  R  -

     AAAGTGAACCCTGAAGTCCAAAGCACCCTCCGGAAGAAGCTCTATTTGCAATAAAAGAGA
 781 ---------+---------+---------+---------+---------+---------+ 840
     TTTCACTTGGGACTTCAGGTTTCGTGGGAGGCCTTCTTCGAGATAAACGTTATTTTCTCT

a    K  V  N  P  E  V  Q  S  T  L  R  K  K  L  Y  L  Q  *  K  R  -

     GGGCAGGGAT
 841 ---------+ 850
     CCCGTCCCTA

a    G  Q  G  -
```

Fig. 1B

```
casp-11  ----------------------------------------------------------------------------------------------------
casp-12  --------------------------------------------------------------------------MAARRTHERDPIYKIKGLAKDMLDGV  26
casp-1   ---------------------------------------------------------------------------MADKILRAKRKQFINSVSIGTINGL  25
casp-3   ----------------------------------------------------------------------------------------------------
casp-7   ----------------------------------------------------------------------------------------------------
casp-6   ----------------------------------------------------------------------------------------------------
casp-8   MDFQSCLDAIAEELGSEDLAALKFLCLDYIPHKKLETIEDAQKLFLRLREKGMLEEGNLSFLKELLFHISRWDLLVNFLDCNREEMVRELRDPRQCPRFL  100
casp-2   ------------------------------------------------MAAPSGRSQSSLHRKGLMAADRRSRILAVCGMHPDHQETLKKNRVVLAKQLL  52
casp-14  ----------------------------------------------------------------------------------------------------

casp-11  ---------------MAENKHPDKPLKVLEQLGKEVL..TEYLEKLVQSNVLKLKEEDKQKFNNAERSDKRWVFV.DAMKKKHSKVGEMLLQTFFSVDPG  82
casp-12  FDDLVEKNVLNGDELLKIGESASFILNKAENLVENFLEKTDMAGKIFAGHIANSQEQLSLQFSNDEDDGPQKICTPSSPSESKRKVEDDEMEVNAGLAHE  126
casp-1   LDELLEKRVLNQEEMDKIKLANITAMDKARDLCDHVSKKGPQASQIFITYICNEDCYLAGILELQSAPSAETFVATEDSKGGHPSSSETKEEQN..KEDG  123
casp-3   --------------------------------------------------------------------------------------------MENNKTSV  8
casp-7   ---------------------------------------------------------------------MTDDQDCAAELEKVDSSSEDGVDAKPDRSSI  31
casp-6   ----------------------------------------------------------------------------------------------------
casp-8   PYRSCSFRLSEEVSELELRSFKFLLNNEIPKCKLEDDLSLLEIFVEMEKRTMLAENNLETLKSICDQVNKSLLGKIEDYERSSTERRMSLEGREELPPSV  200
casp-2   LSELLEHLLEKDIITLEMRELIQAKGGSFSQNVELLNLLPKRGPQAFDAFCEALRETRQGHLEDLLLTTLSDIQHVLPPLSCDYDTSLPFSVCESCPPHK  152
casp-14  ----------------------------------------------------------------------------------------------------

casp-11  SHHGEANLEMEEPEESLNTLKLCSPEEFTRLCREKTQEIYPIKEAN.GRTRKALIICNTEFKHLSLRYGANFDIIGMKGLLEDLGYDVVVKEELTAEGME  181
casp-12  SHLMLTAPHGLQSSEVQDTLKLCPRDQFCKIKTERAKEIYPVMEKE.GRTRLALIICNKKFDYLFDRDNADTDILNMQELLENLGYSVVLKENLTAQEME  225
casp-1   TFPGLTG...........TLKFCPLEKAQKLWKENPSEIYPIMNTT.TRTRLALIICNTEFQHLSPRVGAQVDLREMKLLLEDLGYTVKVKENLTALEMV  211
casp-3   DSKSINNFEVKTIHGSKSVDSGIYLDSSYKMDYPEMGICIIINNKNFHKS...........TGMSSRSGTDVDAANLRETFMNLGKYCVRNKNDLTREDIL  97
casp-7   ISSILLKKKRNASAGPVRTGRDRVPTYLYRMDFQKMGKCIIINNKNFDKA...........TGMDVRNGTDKDAGALFKCFQNLGFEVTVHNDCSCAKMQ  120
casp-6   ---------MTETDGFYKSREVFDPAEQYKMDHKRRGVALIFNHERFFWH...........LTLPERRGTNADRDNLTRRFSDLGFEVKCFNDLRAEELL  80
casp-8   LDEMSLKMAELCDSPREQDSESRTSDKVYQMKNKPRGYCLIINNHDFSKA....REDITQLRKMKDRKGTDCDKEALSKTFKELHFEIVSYDDCTANEIH  296
casp-2   QLRLSTDATEHSLDNGDGPPCLLVKPCTPEFYQAHYQLAYRLQSQPRGLALVLSNVHFTGEKDLEFRSGGDVDHTTLVTLFKLLGYNVHVLHDQTAQEMQ  252
casp-14  -----------------------------MESEMSDPQPLQEERYDMSGARLALTLCVTK...AREGSEVDMEALERMFRYLKFESTMKRDPTAQQFL  66

casp-11  SEMDKFAAL..SEHQTSDSTFLVLMSHGTLHGICGTMHSEKTPDVLQYDTIYQIFNNCHCPGLRDKPKVIIVQACRGGNSGEMWIRESSKPQLCRGVDLP  279
casp-12  TELMQFAGR..PEHQSSDSTFLVFMSHGILEGICGVKHRNKKPDVLHDDTIFKIFNNSNCRSLRNKPKILIMQACRGRYNGTIWV.STNKGIATADTDEE  322
casp-1   KEVKEFAAC..PEHKTSDSTFLVFMSHGIQEGICGTTYSNEVSDILKVDTIFQMMNTLKCPSLKDKPKVIIIQACRGEKQGVVLLKDS.....VRDSEED  304
casp-3   ELMDSVSK...EDHSKRSSFVCVILSHGDEGVIYGTNGP......VELKKLTSFFRGDYCRSLTGKPKLFIIQACRGTELDCGI........ETDS...G  177
casp-7   DLLRKASE...EDHSNSACFACVLLSHGEEDLIYGKDGV......TPIKDLTAHFRGDRCKTLLEKPKLFFIQACRGTELDDGI........QADS...G  200
casp-6   LKIHEVST...SSHIDADCFICVFLSHGEGNHVYAYDAK......IEIQTLTGLFKGDKCQSLVGKPKIFIIQACRGSQHDVPVVPLDMVDHQTDK..LD  169
casp-8   EILEGYQS...ADHKNKDCFICCILSHGDKGVVYGTDGK.....EASIYDLTSYFTGSKCPSLSGKPKIFFIQACRGSNFQKGVPDEAGFEQQNHT..LE  356
casp-2   EKLQNFAQ.LPAHRVTDSVC.VALLSHGVEGGIYGVDGK.....LLQLQEVFRLFDNANCPSLQNKPKMFFIQACRGDETDRGVDQQDGKNHTQSPGCEE  345
casp-14  EELDEFQQTIDNWEEPVSCAFVVLMAHGEEGLLKGEDEK.....MVRLEDLFEVLNNKNCKALRGKPKVYIIQACRGEHRDPGEELRGNEELGGDEELGG  161
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Fig. 2A-1

```
casp-11  RNMEA....DAVKLSHVEKDFIAFYSTTPHHLSYRDKTGGSYFITRLISCFRKHACSCHLFDIFLKVQQSFEKASIHSQMPTIDRATLTRYFYLFPGN--  373
casp-12  RVLSCKW.NNSITKAHVETDFIAFKSSTPHNISWRVGKTGSLFISKLIDCFKKYCWCYHLEEIFRKVQHSFEVPGELTQMPTIERVSMTRYFYLFPGN--  419
 casp-1  FLTDAIFEDDGIKKAHIEKDFIAFCSSTPDNVSWRHPVRGSLFIESLIKHMKEYAWSCDLEDIFRKVRFSFEQPEFRLQMPTADRVTLTKRFYLFPGH--  402
 casp-3  ..TDEEMAC...QKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCSMLKLY..AHKLEFMHILTRVNRKVATEFESFSLDSTFHAKKQIPCIVSMLT  270
 casp-7  ..PINDIDANPRNKIPVEADFLFAYSTVPGYYSWRNPGKGSWFVQALCSILNEH..GKDLEIMQILTRVNDRVARHFESQSCDPRFNEKKQIPCMVSMLT  296
 casp-6  ..NVTQVDAASVYTLPAGADFLMCYSVAEGYYSHRETVNGSWYIQSLCEMLARY..GSSLEFTELLTLVNRKVSQRRVDFCKDPDAIGKKQVPCFASMLT  265
 casp-8  ..VDS...SSHKNYIPDEADFLLGMATVLMCVSYRDPVNGTWYIQSLCQSLRERC.PQGDDILSILTGVNYDVSN.....KDDRRNKGKQMPQPTFTLR  474
 casp-2  ..SDAGKEELMKMRLPTRSDMICGYACLKGNAAMRNTKRGSWYIEALTQVFSERA.C.DMHVADMLVKVNALIKER.EGYAPGTEFHRCKEMSEYCSTLC  440
casp-14  ..DEVAVLKNNPQSIPTYTDTLHIYSTVEGYLSYRHDEKGSGFIQTLTDVFIHKK.G.S..ILELTEEITRLMANT.EVMQEGKP...RKVNPEVQSTLR  251

casp-11  ----------
casp-12  ----------
 casp-1  ----------
 casp-3  KELYFYH-----  277
 casp-7  KELYFSR-----  303
 casp-6  KKLHFCPKPSK-  276
 casp-8  KKL---------  477
 casp-2  QQLYLFPGYPPT  452
casp-14  KKLYLQ------  257
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Fig. 2A-2

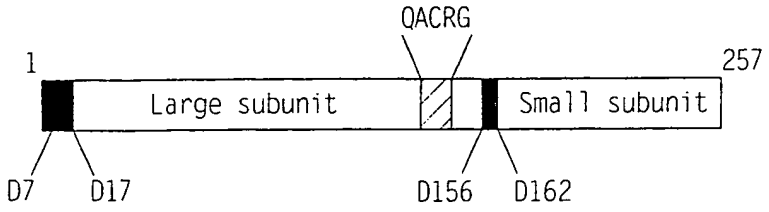


Fig. 2B

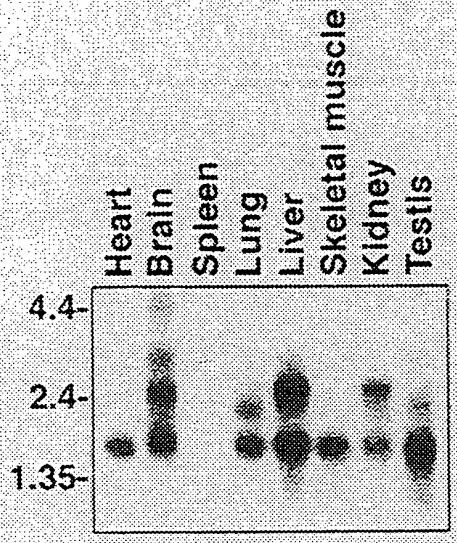
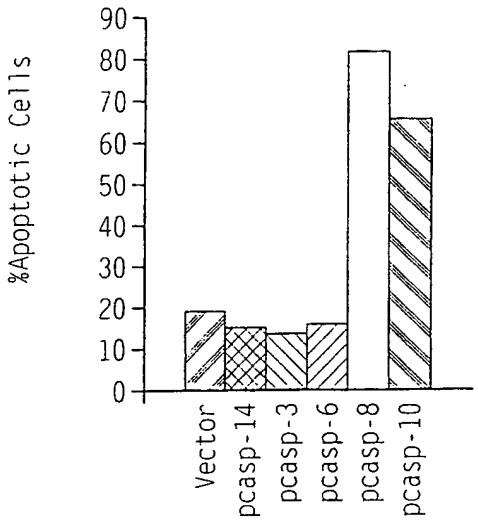

Heart
Brain
Spleen
Lung
Liver
Skeletal muscle
Kidney
Testis
4.4-
2.4-
1.35-

Fig. 3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90
80
70
60
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10
0
Vector
pcasp-14
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pcasp-6
pcasp-8
pcasp-10

Fig. 4

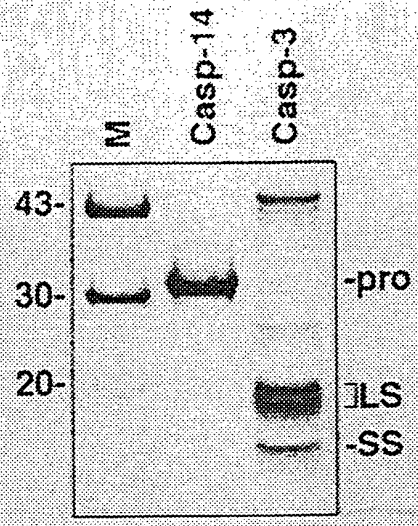
M
Casp-14
Casp-3
43-
30-
20-
-pro
-LS
-SS

Fig. 5A

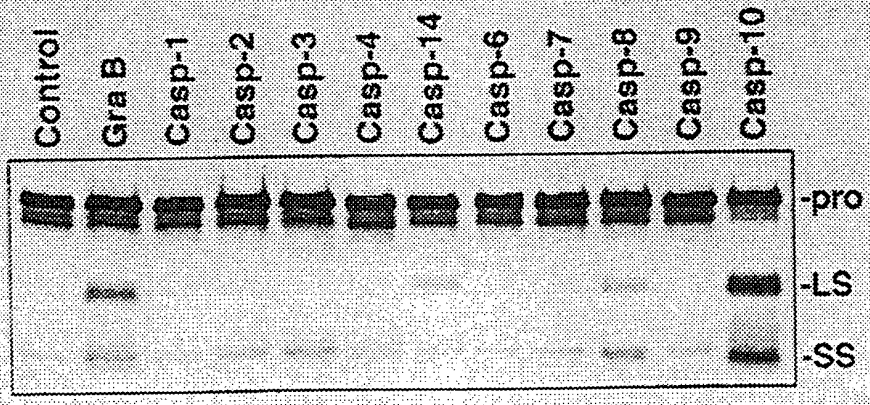
Control
Gra B
Casp-1
Casp-2
Casp-3
Casp-4
Casp-14
Casp-6
Casp-7
Casp-8
Casp-9
Casp-10
-pro
-LS
-SS

Fig. 5B

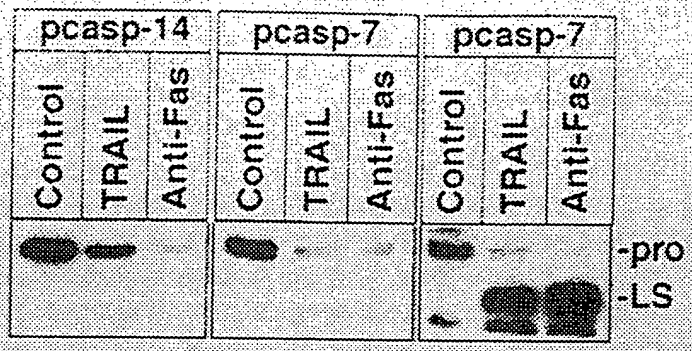
pcasp-14
pcasp-7
pcasp-7
Control
TRAIL
Anti-Fas
Control
TRAIL
Anti-Fas
Control
TRAIL
Anti-Fas
-pro
-LS

Fig. 5C

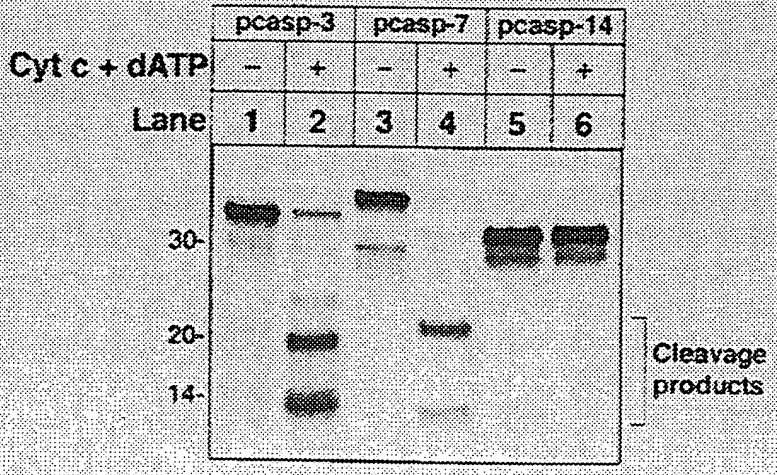
pcasp-3
pcasp-7
pcasp-14
Cyt c + dATP
– + – + – +
Lane
1 2 3 4 5 6
30-
20-
14-
Cleavage products

Fig. 6

```
aggatcagac aagggtgctg agagccggga ctcacaacca aaggagaa atg agc aat  57
                                                      Met Ser Asn
                                                        1

ccg cgg tct ttg gaa gag gag aaa tat gat atg tca ggt gcc cgc ctg  105
Pro Arg Ser Leu Glu Glu Glu Lys Tyr Asp Met Ser Gly Ala Arg Leu
  5                 10                  15

gcc cta ata ctg tgt gtc acc aaa gcc cgg gaa ggt tcc gaa gaa gac  153
Ala Leu Ile Leu Cys Val Thr Lys Ala Arg Glu Gly Ser Glu Glu Asp
 20                  25                  30                  35

ctg gat gct ctg gaa cac atg ttt cgg cag ctg aga ttc gaa agc acc  201
Leu Asp Ala Leu Glu His Met Phe Arg Gln Leu Arg Phe Glu Ser Thr
                 40                  45                  50

atg aaa aga gac ccc act gcc gag caa ttc cag gaa gag ctg gaa aaa  249
Met Lys Arg Asp Pro Thr Ala Glu Gln Phe Gln Glu Glu Leu Glu Lys
             55                  60                  65

ttc cag cag gcc atc gat tcc cgg gaa gat ccc gtc agt tgt gcc ttc  297
Phe Gln Gln Ala Ile Asp Ser Arg Glu Asp Pro Val Ser Cys Ala Phe
         70                  75                  80

gtg gta ctc atg gct cac ggg agg gaa ggc ttc ctc aag gga gaa gat  345
Val Val Leu Met Ala His Gly Arg Glu Gly Phe Leu Lys Gly Glu Asp
     85                  90                  95

ggg gag atg gtc aag ctg gag aat ctc ttc gag gcc ctg aac aac aag  393
Gly Glu Met Val Lys Leu Glu Asn Leu Phe Glu Ala Leu Asn Asn Lys
100                 105                 110                 115

aac tgc cag gcc ctg cga gct aag ccc aag gtg tac atc ata cag gcc  441
Asn Cys Gln Ala Leu Arg Ala Lys Pro Lys Val Tyr Ile Ile Gln Ala
                120                 125                 130

tgt cga gga gaa caa agg gac ccc ggt gaa aca gta ggt gga gat gag  489
Cys Arg Gly Glu Gln Arg Asp Pro Gly Glu Thr Val Gly Gly Asp Glu
            135                 140                 145

att gtg atg gtc atc aaa gac agc cca caa acc atc cca aca tac aca  537
Ile Val Met Val Ile Lys Asp Ser Pro Gln Thr Ile Pro Thr Tyr Thr
        150                 155                 160
```

Fig. 7A

```
gat gcc ttg cac gtt tat tcc acg gta gag gga tac atc gcc tac cga  585
Asp Ala Leu His Val Tyr Ser Thr Val Glu Gly Tyr Ile Ala Tyr Arg
    165                 170                 175

cat gat cag aaa ggc tca tgc ttt atc cag acc ctg gtg gat gtg ttc  633
His Asp Gln Lys Gly Ser Cys Phe Ile Gln Thr Leu Val Asp Val Phe
180                 185                 190                 195

acg aag agg aaa gga cat atc ttg gaa ctt ctg aca gag gtg acc cgg  681
Thr Lys Arg Lys Gly His Ile Leu Glu Leu Leu Thr Glu Val Thr Arg
                200                 205                 210

cgg atg gca gaa gca gag ctg gtt caa gaa gga aaa gca agg aaa acg  729
Arg Met Ala Glu Ala Glu Leu Val Gln Glu Gly Lys Ala Arg Lys Thr
            215                 220                 225

aac cct gaa atc caa agc acc ctc cgg aaa cgg ctg tat ctg cag tag  777
Asn Pro Glu Ile Gln Ser Thr Leu Arg Lys Arg Leu Tyr Leu Gln
        230                 235                 240
```

Fig. 7B

```
aggatcagac aagggtgctg agagccggga ctcacaacca aaggagaa atg agc aat   57
                                                      Met Ser Asn
                                                        1

ccg cgg tct ttg gaa gag gag aaa tat gat atg tca ggt gcc cgc ctg   105
Pro Arg Ser Leu Glu Glu Glu Lys Tyr Asp Met Ser Gly Ala Arg Leu
  5                  10                  15

gcc cta ata ctg tgt gtc acc aaa gcc cgg gaa ggt tcc gaa gaa gac   153
Ala Leu Ile Leu Cys Val Thr Lys Ala Arg Glu Gly Ser Glu Glu Asp
 20                  25                  30                  35

ctg gat gct ctg gaa cac atg ttt cgg cag ctg aga ttc gaa agc acc   201
Leu Asp Ala Leu Glu His Met Phe Arg Gln Leu Arg Phe Glu Ser Thr
                 40                  45                  50

atg aaa aga gac ccc act gcc gag caa ttc cag gaa gag ctg gaa aaa   249
Met Lys Arg Asp Pro Thr Ala Glu Gln Phe Gln Glu Glu Leu Glu Lys
             55                  60                  65

ttc cag cag gcc atc gat tcc cgg gaa gat ccc gtc agt tgt gcc ttc   297
Phe Gln Gln Ala Ile Asp Ser Arg Glu Asp Pro Val Ser Cys Ala Phe
         70                  75                  80

gtg gta ctc atg gct cac ggg agg gaa ggc ttc ctc aag gga gaa gat   345
Val Val Leu Met Ala His Gly Arg Glu Gly Phe Leu Lys Gly Glu Asp
     85                  90                  95

ggg gag atg gtc aag ctg gag aat ctc ttc gag gcc ctg aac aac aag   393
Gly Glu Met Val Lys Leu Glu Asn Leu Phe Glu Ala Leu Asn Asn Lys
100                 105                 110                 115

aac tgc cag gcc ctg cga gct aag ccc aag gtg tac atc ata cag gcc   441
Asn Cys Gln Ala Leu Arg Ala Lys Pro Lys Val Tyr Ile Ile Gln Ala
                120                 125                 130

tgt cga gga gaa caa agg gac ccc ggt gaa aca gta ggt gga gat gag   489
Cys Arg Gly Glu Gln Arg Asp Pro Gly Glu Thr Val Gly Gly Asp Glu
            135                 140                 145

att gtg atg gtc atc aaa gac agc cca caa acc atc cca aca tac aca   537
Ile Val Met Val Ile Lys Asp Ser Pro Gln Thr Ile Pro Thr Tyr Thr
        150                 155                 160
```

Fig. 8A

```
gat gcc ttg cac gtt tat tcc acg gta gag gga ccc acg ccc ttc cag   585
Asp Ala Leu His Val Tyr Ser Thr Val Glu Gly Pro Thr Pro Phe Gln
    165                 170                 175

gat ccc ctc tac cta ccc tct gaa gct ccc ccg aac cca cct ctc tgg   633
Asp Pro Leu Tyr Leu Pro Ser Glu Ala Pro Pro Asn Pro Pro Leu Trp
180                 185                 190                 195

aat tcc cag gat aca tcg cct acc gac atg atc aga aag gct cat gct   681
Asn Ser Gln Asp Thr Ser Pro Thr Asp Met Ile Arg Lys Ala His Ala
                200                 205                 210

tta tcc aga ccc tgg tgg atg tgt tca cga aga gga aag gac ata tct   729
Leu Ser Arg Pro Trp Trp Met Cys Ser Arg Arg Gly Lys Asp Ile Ser
            215                 220                 225

tgg aac ttc tgacagaggt gacccggcgg atggcagaag cagagctggt          778
Trp Asn Phe
        230

tcaagaagga aaagcaagga aaacgaaccc tgaaatccaa agcaccctcc ggaaacggct 838

gtatctgcag tag                                                   851
```

Fig. 8B

```
aggatcagac aagggtgctg agagccggga ctcacaacca aaggagaa atg agc aat  57
                                                      Met Ser Asn
                                                        1

ccg cgg tct ttg gaa gag gag aaa tat gat atg tca ggt gcc cgc ctg  105
Pro Arg Ser Leu Glu Glu Glu Lys Tyr Asp Met Ser Gly Ala Arg Leu
  5                  10                  15

gcc cta ata ctg tgt gtc acc aaa gcc cgg gaa ggt tcc gaa gaa gaa  153
Ala Leu Ile Leu Cys Val Thr Lys Ala Arg Glu Gly Ser Glu Glu Glu
 20                  25                  30                  35

gag ctg gaa aaa ttc cag cag gcc atc gat tcc cgg gaa gat ccc gtc  201
Glu Leu Glu Lys Phe Gln Gln Ala Ile Asp Ser Arg Glu Asp Pro Val
                 40                  45                  50

agt tgt gcc ttc gtg gta ctc atg gct cac ggg agg gaa ggc ttc ctc  249
Ser Cys Ala Phe Val Val Leu Met Ala His Gly Arg Glu Gly Phe Leu
             55                  60                  65

aag gga gaa gat ggg gag atg gtc aag ctg gag aat ctc ttc gag gcc  297
Lys Gly Glu Asp Gly Glu Met Val Lys Leu Glu Asn Leu Phe Glu Ala
         70                  75                  80

ctg aac aac aag aac tgc cag gcc ctg cga gct aag ccc aag gtg tac  345
Leu Asn Asn Lys Asn Cys Gln Ala Leu Arg Ala Lys Pro Lys Val Tyr
 85                  90                  95

atc ata cag gcc tgt cga gga gaa caa agg gac ccc ggt gaa aca gta  393
Ile Ile Gln Ala Cys Arg Gly Glu Gln Arg Asp Pro Gly Glu Thr Val
100                 105                 110                 115

ggt gga gat gag att gtg atg gtc atc aaa gac agc cca caa acc atc  441
Gly Gly Asp Glu Ile Val Met Val Ile Lys Asp Ser Pro Gln Thr Ile
                120                 125                 130

cca aca tac aca gat gcc ttg cac gtt tat tcc acg gta gag gga tac  489
Pro Thr Tyr Thr Asp Ala Leu His Val Tyr Ser Thr Val Glu Gly Tyr
            135                 140                 145

atc gcc tac cga cat gat cag aaa ggc tca tgc ttt atc cag acc ctg  537
Ile Ala Tyr Arg His Asp Gln Lys Gly Ser Cys Phe Ile Gln Thr Leu
        150                 155                 160
```

Fig. 9A

```
gtg gat gtg ttc acg aag agg aaa gga cat atc ttg gaa ctt ctg aca  585
Val Asp Val Phe Thr Lys Arg Lys Gly His Ile Leu Glu Leu Leu Thr
    165                 170                 175

gag gtg acc cgg cgg atg gca gaa gca gag ctg gtt caa gaa gga aaa  633
Glu Val Thr Arg Arg Met Ala Glu Ala Glu Leu Val Gln Glu Gly Lys
180                 185                 190                 195

gca agg aaa acg aac cct gaa atc caa agc acc ctc cgg aaa cgg ctg  681
Ala Arg Lys Thr Asn Pro Glu Ile Gln Ser Thr Leu Arg Lys Arg Leu
                200                 205                 210

tat ctg cag tag                                                 693
Tyr Leu Gln
```

Fig. 9B

```
1   MESEMSDPQPLQEERYDMSGARLALTLCVTKAREGSEVDMEALERMFRYL  50
        ||.|. |:||:||||||||||| |||||||||| |::||| ||| |
1   ....MSNPRSLEEEKYDMSGARLALILCVTKAREGSEEDLDALEHMFRQL  46

51  KFESTMKRDPTAQQFLEELDEFQQTIDNWEEPVSCAFVVLMAHGEEGLLK 100
    :||||||||||:|| |||:.||| ||. |:||||||||||||| || ||
47  RFESTMKRDPTAEQFQEELEKFQQAIDSREDPVSCAFVVLMAHGREGFLK  96

101 GEDEKMVRLEDLFEVLNNKNCKALRGKPKVYIIQACRGEHRDPGEELRGN 150
    ||| .||:||.||| |||||| ||| |||||||||||| ||||
97  GEDGEMVKLENLFEALNNKNCQALRAKPKVYIIQACRGEQRDPG.....  140

151 EELGGDEELGGDEVAVLKNNPQSIPTYTDTLHIYSTVEGYLSYRHDEKGS 200
    | .|||      | |:|..||.||||| ||:||||||:.||||:|||
141 ETVGGDE.....IVMVIKDSPQTIPTYTDALHVYSTVEGYIAYRHDQKGS 185

201 GFIQTLTDVFIHKKGSILELTEEITRLMANTEVMQEGKPRKVNPEVQSTL 250
    ||||| ||| :|| |||| |:|| || |..|||| || |||:||||
186 CFIQTLVDVFTKRKGHILELLTEVTRRMAEAELVQEGKARKTNPEIQSTL 235

251 RKKLYLQ* 257
    ||:||||
236 RKRLYLQ* 242
```

Fig. 10

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 99/25523

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/57 C12N9/64 C12N5/10 C07K16/40 C12Q1/37

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN DE CRAEN M ET AL: "Identification of a new caspase homologue: caspase - 14" CELL DEATH AND DIFFERENTIATION,GB,EDWARD ARNOLD, OXFORD, vol. 5, no. 10, October 1998 (1998-10), pages 838-846, XP002123472 ISSN: 1350-9047	1-18, 21-55
Y	the whole document ---	19,20
Y	SCAFFIDI CARSTEN ET AL: "FLICE is predominantly expressed as two functionally active isoforms, caspase-8/a and caspase-8/b." JOURNAL OF BIOLOGICAL CHEMISTRY 1997, vol. 272, no. 43, 1997, pages 26953-26958, XP002132494 ISSN: 0021-9258	39-42
A	the whole document ---	39-42
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2000	22/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van der Schaal, C

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/25523

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
Y	WO 96 25945 A (UNIV JEFFERSON) 29 August 1996 (1996-08-29)	39-42
A	the whole document	39-42

P,X, L	WO 99 10504 A (ALNEMRI EMAD S ;FERNANDES ALNEMRI TERESA (US); IDUN PHARMACEUTICAL) 4 March 1999 (1999-03-04)	1-38, 43-55
Y	L: priority	39-42
	the whole document	

E	WO 00 04169 A (CRAEN MARC VAN DE ;VLAAMS INTERUNIV INST BIOTECH (BE); DECLERCQ WI) 27 January 2000 (2000-01-27)	1-18, 21-55
	the whole document	

P,A	WO 99 23106 A (HUMAN GENOME SCIENCES INC ;NI JIAN (US); RUBEN STEVEN M (US)) 14 May 1999 (1999-05-14)	

P,X	AHMAD M ET AL: "Identification and characterization of murine caspse-14, a new member of the caspase familiy" CANCER RESEARCH, vol. 58, 15 November 1998 (1998-11-15), pages 5201-5205, XP002132503	1-18, 21-38, 43-55
Y	the whole document	19,20, 39-42

P,X	HU S ET AL: "Caspase - 14 is a novel developmentally regulated protease" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 273, no. 45, 15 November 1998 (1998-11-15), pages 29648-29653, XP002123473 ISSN: 0021-9258	1-18, 21-38, 43-55
Y	the whole document	19,20, 39-42

Y	COHEN G: "Caspase: the executioners of apoptosis" BIOCHEMICAL JOURNAL,GB,PORTLAND PRESS, LONDON, vol. 326, 15 August 1997 (1997-08-15), pages 1-16, XP002091927 ISSN: 0264-6021	19,20
	the whole document	

	-/--	

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 99/25523

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
Y	PIERARD L ET AL: "MUTANT AND CHIMERIC RECOMBINANT PLASMINOGEN ACTIVATORS. PRODUCTION IN EULARYOTIC CELLS AND PRELIMINARY CHARACTERIZATION" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 262, no. 24, 25 August 1987 (1987-08-25), pages 11771-11778, XP002064764 ISSN: 0021-9258 the whole document	19,20
Y	WO 95 24427 A (UNIV EMORY ;LOLLAR JOHN S (US); RUNGE MARSCHALL S (US)) 14 September 1995 (1995-09-14) the whole document	19,20

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 25523

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additonal sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 99/25523

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found mulitple (groups of) inventions in this international application, as follows:

1.Claims:2-5, 13, 14, 17, 18, 39-44, 46 completely,
Claims 1, 6-12, 15, 16, 49-51 partially.

Human caspase 14 and nucleotides encoding it.

2.Claims: 19, 20

Fusion polypeptides between small and large subunits of caspase-14 and large and small subunits of caspase 1-10 or ced-3.

3.Claims 21-24

Anti caspase-14 antibodies.

4. Claims: 25-38

Method of identifying compounds that modulate caspase-14.

5.Claims 45, 47, 48, 52-55 Completely,
Claims 1, 6-12, 15, 16, 49-51 partially.

Murine caspase-14 and nuleotides encoding it.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter ɔnal Application No
PCT/US 99/25523

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9625945 A	29-08-1996	US 5856169 A	05-01-1999
		CA 2211541 A	29-08-1996
		EP 0814832 A	07-01-1998
		JP 11501507 T	09-02-1999
WO 9910504 A	04-03-1999	AU 9121998 A	16-03-1999
WO 0004169 A	27-01-2000	NONE	
WO 9923106 A	14-05-1999	AU 9673898 A	24-05-1999
WO 9524427 A	14-09-1995	US 5663060 A	02-09-1997
		AU 693837 B	09-07-1998
		AU 1209695 A	25-09-1995
		CA 2185327 A	14-09-1995
		EP 0749444 A	27-12-1996
		JP 10500282 T	13-01-1998
		US 5744446 A	28-04-1998
		US 5888974 A	30-03-1999
		US 5859204 A	12-01-1999